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1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

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(54) Title: METHOD FOR PREPARING POLYCATION BASED BIOCONJUGATES SUITABLE FOR TRANSPORTING DIFFERENT KINDS OF ACTIVE SUBSTANCES WITHIN THE BODY

$$H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}\underset{\displaystyle [(i)Mx] \cdot [(k)Mx] - NH}{\underset{|}{CH}}\text{-}CO]_rOH \qquad (I)$$

(57) Abstract: The invention relates to new polycation bioconjugates and to a method for producing them. New polycation bioconjugates according to the invention are characterized by that the comprised polycations are capable of transporting active substances of different types in the mammal organism, i.e. for functioning as carrier molecules, thus are able to enhance the biological effectiveness of the transported molecules, and consequently they can, for example, favourably inhibit malignant cell proliferation, or they possess antimicrobial effect, or are suitable for transportation of genes. A further characteristic of the polycation bioconjugates according to the invention is that each of them contains isopolypeptide carrier molecules, bearing free α-amino group, as a common characteristic structural element. Enhancer molecules - same or different - having appropriate binding functions are coupled by chemical bonds directly and/or indirectly through connecting molecules - that may be identical or different ones - to the carrier molecule. Hence the polycation bioconjugates synthetized according to the invention are of general formula (I) wherein: "r" is a mean value between 20 and 400, "m"=0, 1, 2, 3, ...k, "[(k)Mx]" designates enhancer molecules and/or connecting molecules conjugated by covalent (=k) bonds to the isopolypeptide polycation carrier molecule, and "[(i)Mx]" designates enhancer molecules conjugated by ionic (=i) bonds to the isopolypeptide polycation carrier molecule, whereas the said enhancer molecules and connecting molecules having appropriate functional groups for conjugation may either be identical ones or of (two or more i.e "x") different kinds, and the enhancer molecules can be conjugated directly and/or indirectly through a connecting molecule.

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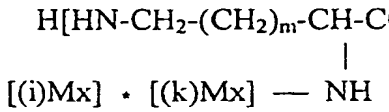
METHOD FOR PREPARING POLYCATION BASED BIOCONJUGATES SUITABLE FOR TRANSPORTING DIFFERENT KIND OF ACTIVE SUBSTANCES WITHIN THE BODY

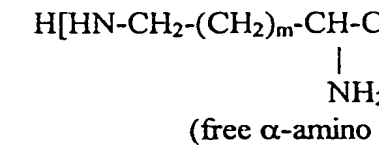
Subject of the present invention is the preparation of polycation based bioconjugates that are suitable for transporting active substances of different type within the body, that is for functioning as carriers.

New polycation bioconjugates according to the invention are prepared by coupling [(k)Mx] and/or [(i)Mx] molecules, bearing functional groups appropriate for conjugation – which may either be identical ones or of (two or more i.e. "x") different kind – to a given representative of isopolypeptide polycations, having free α-amino groups, as carrier molecules, by chemical bonds; and the bioconjugates synthetized this way can be described by the general formula (I):



(I)

and within the polycation bioconjugates there are isopolypeptide polycation carrier molecules (further on: carrier molecules), having free α-amino groups, that can be described by the general formula (I/a):



(I/a)

(free α-amino group)

and in each carrier molecule of general formula (I/a) there are monomeres of the same configuration (i.e. either D-, or L-), and the individual monomeres are not linked together by their amino groups in the α-positions, but by their in other amino groups (i.e. in β-, γ-, δ-, ε etc.) positions, according to the value of "m", and their structures are therefore divergent from those of the polypeptides build up by customary α-amino-peptide bonds, generally occurring in mammal organisms;

wherein:

"r" is a mean value between 20 and 400;
"m" = 0, 1, 2, 3, ... k;
"[(k)Mx]" designates enhancer molecules and/or connecting molecules conjugated by covalent (= k) bonds to the isopolypeptide polycation carrier molecule, and
"[(i)Mx]" designates enhancer molecules conjugated by ionic (= i) bonds to the isopolypeptide polycation carrier molecule, whereas the said enhancer molecules and connecting molecules having appropriate functional groups for conjugation may either be identical ones or of (two or more i.e. "x") different kind and the enhancer molecules can be conjugated

- directly and/or
- indirectly through a connecting molecule,

and further the joint occurrence of [(k)Mx] and [(i)Mx] within the same polycation bioconjugate is symbolized by [(k/i)Mx]. On the basis of the general formula (I) of the new polycation bioconjugates according to the invention further molecules of general formulae (II), (III), (IV),

(VI), (VII), (IX), (X), (XI) and of schematic formulae (V), (VIII), (IX/a), (X/a), (XI/a) can be derived.

In case the [Ex_i] enhancer molecules - which may either be identical ones or of (two or more i.e. "x") different kind - are directly conjugated to a given representative of carrier molecules of general formula (I/a), by covalent bonds, then:

$$[(k)Mx] = [Ex_i]_{p1},$$

and the new polycation bioconjugates are being described by the general formula (II):

$$H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH(NH\text{-}[Ex_i]_{p1})\text{-}CO]_rOH \qquad \textbf{(II)}$$

In case the [(-)Cx_j] connecting molecules of exclusively anionic character – which may either be identical ones or of (two or more i.e. "x") different kind – are conjugated to a given representative of carrier molecules of general formula (I/a), by covalent bonds, an additional possibility arises to establish ionic bonds with cations – which may either be identical ones or of (two or more i.e. "x") different kind – and then:

$$[(k)Mx] = [(-)Cx_j]_{p2},$$

and the new conjugates are being described by the general formula (III):

$$H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH(NH\text{-}[(-)Cx_j]_{p2})\text{-}CO]_rOH \qquad \textbf{(III)}$$

In case the [Ex_{ck}] enhancer molecules – which may either be identical ones or of (two or more i.e. "x") different kind – are indirectly conjugated by covalent bonds to a given representative of carrier molecules of general formula (I/a), through [Cx_{ck}] connecting molecules – which may also be either identical ones or of (two or more i.e. "x") different kind – then:

$$[(k)Mx] = [Cx_{ck}\text{-}Ex_{ck}]_{p3},$$

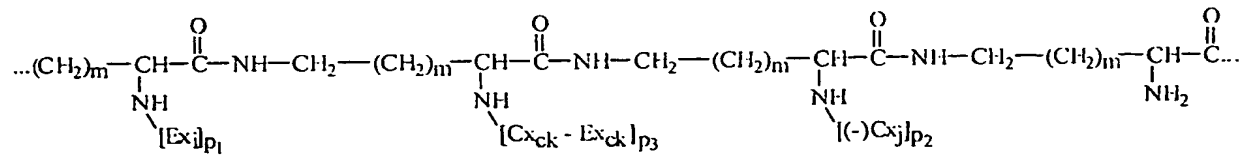
and the new polycation bioconjugates are being described by the general formula (IV):

$$H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH(NH\text{-}[Cx_{ck}\text{-}Ex_{ck}]_{p3})\text{-}CO]_rOH \qquad \textbf{(IV)}$$

In the case when [Ex_i] and/or [Cx_{ck}-Ex_{ck}] enhancer molecules and/or [(-)Cx_j] connecting molecules of anionic character are also conjugated to a given representative of carrier molecules of general formula (I/a), and furthermore from among "p_1, p_2 and p_3" the value of at least two are greater than 0, then:

$$[(k)Mx] = [Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ck}]_{p3} + [(-)Cx_j]_{p2},$$

and the new polycation bioconjugates are being described by the schematic formula (V):



(V)

wherein:

"Ex" in $[Ex_i]_{p1}$ designates the Ex enhancer molecules of different ("x") kind conjugated directly to a given representative of carrier molecules of general formula (**I/a**), by covalent bonds, and

"i" indicates whether the Ex enhancer molecules, conjugated to the given carrier molecule by covalent bonds, are identical ones (i = 1), or they are of different kind, of number "i" (i = 2, 3,... "x" kind); and

"(-)Cx" in $[(-)Cx_j]_{p2}$ designates (-)Cx connecting molecules of exclusively anionic character, of different ("x") kind conjugated to a given representative of carrier molecules of general formula (**I/a**) by covalent bonds, in order to make it capable for establishing ionic bonds with cations, and

"j" indicates whether the (-)Cx connecting molecules, conjugated to the given carrier molecule by covalent bonds, are identical ones (j = 1), or they are of different kind, of number "j" (j = 2, 3,... "x"); and

"Cx-Ex" in $[Cx_{ck}\text{-}Ex_{ck}]_{p3}$ designates the Ex enhancer molecules of different ("x") kind, conjugated by covalent bonds indirectly, through Cx connecting molecules of different ("x") kind, and these Cx molecules are also conjugated by covalent bonds to a given representative of carrier molecules of general formula (**I/a**), and

"ck" indicates whether the Cx connecting molecules, conjugated to a given carrier molecule by covalent bonds, are identical ones (ck = 1), or they are of different kind, of the number "ck" (ck = 2, 3, ... "x"), and these Cx connecting molecules – practically depending on the structure of the Ex enhancer molecules – may be neutral and/or of anionic and/or of cationic character,

"ek" indicates whether the Ex enhancer molecules, conjugated to a given carrier molecule indirectly through "Cx" connecting molecules by covalent bonds, are identical ones (ek = 1), or they are of different kind, of the number "ck" (ek = 2, 3,..."x").

Furthermore the degree of saturation in % of a given representative of carrier moleculea of general formula (**I/a**) by $[Ex_i]_{p1}$ and/or $[Cx_{ck}\text{-}Ex_{ck}]_{p3}$ enhancer molecules and/or $[(-)Cx_j]_{p2}$ connecting molecules are given by the different values of "p_1, p_2 and p_3", whereas the summarized value of "$p_1 + p_2 + p_3$" within one given polycation bioconjugate is > 0 and ≤ 100; whereby the ratio between the free (not involved in peptide bonds) and bound NH_2 -groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates; and thus

"p_1" indicates a degree of saturation in % of a carrier molecule of general formula (**I/a**) with $[Ex_i]$ enhancer molecules,

"p_2" indicates a degree of saturation in % of a carrier molecule of general formula (**I/a**) with $[(-)Cx_j]$ connecting molecules of exclusively anionic character,

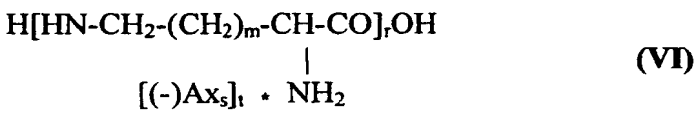
"p_3" indicates a degree of saturation in % of a carrier molecule of general formula (**I/a**) with $[Cx_{ck}\text{-}Ex_{ck}]$ enhancer molecules which are bound to connecting molecules,

and on the basis of the above, in the schematic formula (V) "$p_1 + p_2 + p_3$" > 0 and ≤ 100, and from among "p_1, p_2 and p_3" the value of at least two are greater than 0; further in a given polycation bioconjugate, the Ex molecules in [Ex_i] and the (-)Cx molecules in [(-)Cx_j] are not necessarily identical with those Ex and Cx molecules occurring in [Cx_{ck}-Ex_{ek}], which divergence is symbolized by "x", and will be dealt with later at the examples of suitably selected enhancer molecules, further "r" and "m" have the same meaning as in general formula (I).

In case the [(-)Ax_s] enhancer molecules of anionic character – which may either be identical ones or of (two or more i.e. "x") different kind – are directly conjugated by ionic bonds to the free α-amino groups of a given representative of carrier molecules of general formula (I/a), then:

$$[(i)Mx] = [(-)Ax_s]_t,$$

and the new polycation bioconjugates are being described by the general formula (VI):

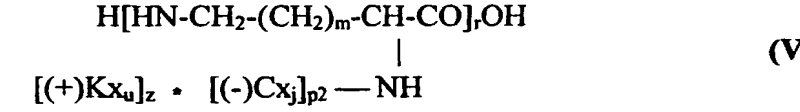


(VI)

In case the [(+)Kx_u] enhancer molecules of cationic character – which may either be identical ones or of (two or more i.e. "x") different kind – are conjugated indirectly through [(-)Cx_j] connecting molecules of exclusively anionic character – which may either be identical ones or of (two or more i.e. "x") different kind – by ionic bonds to a given representative of conjugates of general formula (III), then:

$$[(k)Mx] \cdot [(i)Mx] = [(k/i)Mx] = [(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z,$$

and the new polycation bioconjugates are being described by the general formula (VII):

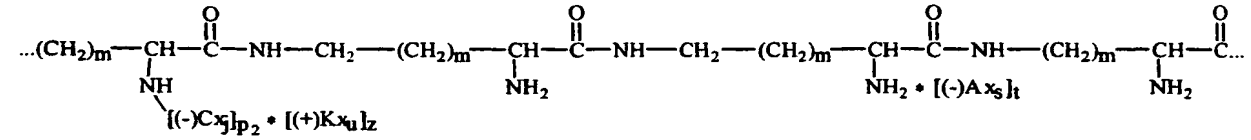


(VII)

In case additional [(-)Ax_s] enhancer molecules of anionic character – which may either be identical ones or of (two or more i.e. "x") different kind – are conjugated directly by ionic bonds to the free α-amino groups of a given representative of polycation bioconjugates of general formula (VII), then:

$$[(k/i)Mx] = \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\} \cdot [(-)Ax_s]_t,$$

and the new polycation bioconjugates are being described by the schematic formula (VIII):



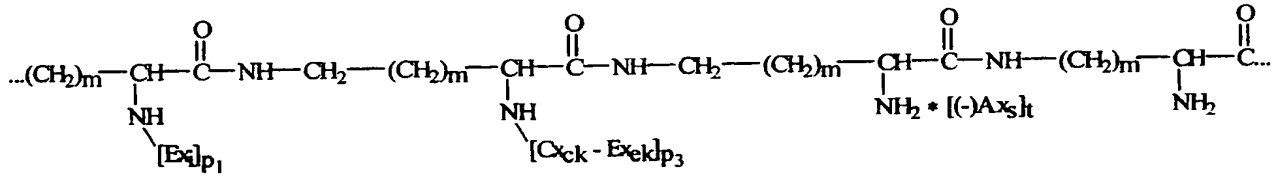
(VIII)

In case additional [(-)Ax_s] enhancer molecules of anionic character – which may either be identical ones or of (two or more i.e. "**x**") different kind – are conjugated by ionic bonds to the free α-amino groups of a given representative of polycation bioconjugates of general formula (**II**) or (**IV**) or of schematic formula (**V**), then:

$$[(k/i)Mx] = [Ex_i]_{p1} * [(-)Ax_s]_t \text{ or } [Cx_{ck}\text{-}Ex_{ek}]_{p3} * [(-)Ax_s]_t \text{ or } [Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} * [(-)Ax_s]_t,$$

and the new polycation bioconjugates are being described by the general formula (**IX**), or by the schematic formula (**IX/a**):

$$[(-)Ax_s]_t \cdot \begin{array}{c} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ | \\ [(k)Mx]\text{—}NH \end{array} \qquad \textbf{(IX)}$$



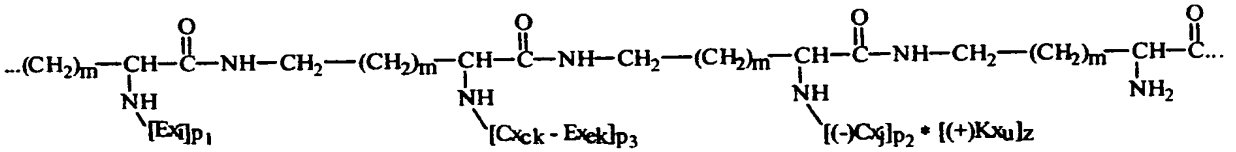
(IX/a)

In case a given representative of polycation bioconjugates of schematic formula (**V**), in which there are [(-)Cx_j] connecting molecules of anionic character – which may also be either identical ones or of (two or more i.e. "**x**") different kind – and it thus gains partially anionic character, so that additional [(+)Kx_u] enhancer molecules of cationic character – which may either be identical ones or of (two or more i.e. "**x**") different kind – can be conjugated by ionic bonds to it, then:

$$\begin{aligned} [(k/i)Mx] = {} & [Ex_i]_{p1} + & & \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} \text{ or} \\ & [Cx_{ck}\text{-}Ex_{ek}]_{p3} + & & \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} \text{ or} \\ & [Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} + & & \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\}, \end{aligned}$$

and the polycation bioconjugates are being described by the general formula (**X**), or by the schematic formula (**X/a**):

$$\begin{array}{c} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ | \\ \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} + [(k)Mx]\text{—}NH \end{array} \qquad \textbf{(X)}$$

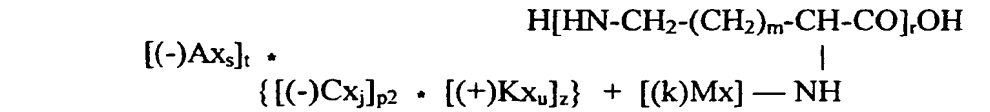


(X/A)

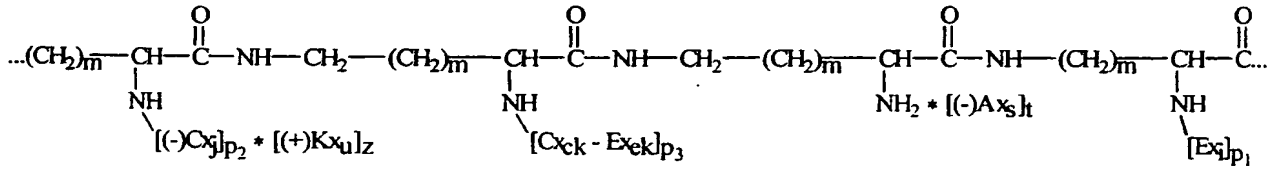
In case additional [(-)Ax_s] enhancer molecules of anionic character – which may either be identical ones or of (two or more i.e. "**x**") different kind – are conjugated directly by ionic bonds to the free α-amino groups of a given representative of polycation bioconjugates of general formula (**X**), then:

$$[(k/i)Mx] = [Ex_i]_{p1} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} * [(-)Ax_s]_t \text{ or}$$
$$[Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} * [(-)Ax_s]_t \text{ or}$$
$$[Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} * [(-)Ax_s]_t,$$

and the polycation bioconjugates are being described by the general formula (**XI**), or by the schematic formula (**XI/a**):



(XI)



(XI/a)

wherein:

"(-)Ax" in $[(-)Ax_s]_t$ designates the (-)Ax enhancer molecules of anionic character, of different ("**x**") kind conjugated directly to a given representative of carrier molecules of general formula (**I/a**), by ionic bonds, and

"**s**" indicates whether the (-)Ax anionic/polyanionic molecules, conjugated to a given polycation carrier molecule by ionic bonds, are identical ones (**s** = 1), or, they are of different kind, of number "**s**" (**s** = 2, 3,..... "**x**" kind), and

"(+)Kx" in $[(+)Kx_u]_z$ designates (+)Kx enhancer molecules of different ("**x**") kind of cationic character that are conjugated indirectly by ionic bonds, through $[(-)Cx_j]$ connecting molecules of different ("**x**") kind of anionic character, to a given representative of carrier molecules of general formula (**I/a**), that is essentially to a conjugate of general formula (**III**), and

"**u**" indicates whether the (+)Kx cations and/or polycations, conjugated to a given compound of general formula (**III**) by ionic bonds, are identical ones (**u** = 1), or they are of different kind of number "**u**" (**u** = 2, 3,..... "**x**" kind), and furthermore

"**t**" indicates a degree of saturation in % of a carrier molecule of general formula (**I/a**) with $[(-)Ax_s]$ enhancer molecules, and

"**z**" indicates a degree of saturation in % of a polycation bioconjugate general formula (**I**), or a carrier molecule of general formula (**I/a**) with $[(+)Kx_u]$ enhancer molecules, which are conjugated indirectly through $[(-)Cx_j]$ connecting molecules of anionic character, and

"**t**" in general formula (**VI**), and

"**z**" in general formula (**VII**), and

"**t**"+"**z**" in schematic formula (**VIII**), and

"**t**"+"p_1" or "**t**"+"p_3" or "**t**"+"p_1"+"p_3" in general formula (**IX**) and in schematic formula (**IX/a**), and

"**z**"+"p_1" or "**z**"+"p_3" or "**z**"+"p_1"+"p_3" in general formula (**X**) and in schematic formula (**X/a**), and

"**t**"+"**z**"+"p_1" or "**t**"+"**z**"+"p_3" or "**t**"+"**z**"+"p_1"+"p_3" in general formula (**XI**) and in schematic formula (**XI/a**), and the value of each of these sums is between >0 and ≤100; furthermore

according to these the NH_2-groups which are not saturated by "**t**" and/or "**z**" and/or "$\mathbf{p_1}$" and/or "$\mathbf{p_3}$" will remain free, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2-groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates, whereas "**z**" indicates the degree of saturation of carrier molecules with $[(+)\mathbf{Kx}_u]$ cationic enhancer molecules that are conjugated indirectly through $[(-)\mathbf{Cx}_j]$ connecting molecules, therefore "**z**" = "$\mathbf{p_2}$"; furthermore

"**r**" and "**m**" and "[(k)**Mx**]" have the same meaning as in general formula (**I**),
"$[\mathbf{Ex}_i]_{p1}$" has the same meaning as in general formula (**II**),
"$[(-)\mathbf{Cx}_j]_{p2}$" has the same meaning as in general formula (**III**),
"$[\mathbf{Cx}_{ck}\text{-}\mathbf{Ex}_{ek}]_{p3}$" has the same meaning as in general formula (**IV**).

Novelty of the polycation bioconjugates of general formula (**I**), subject of the present invention, consists in that there are isopolypeptides having free α-amino groups, as polycation carrier molecules in them, the synthesis of which is being carried out by coupling the diamino-monocarbonic acid monomers that build up these isopolypeptides, not by their amino groups in α-positions, but their amino groups in other (β-, γ-, δ-, ε-...etc.) positions, and the method used for preparation of these carrier molecules has been disclosed in the patent specification HU 202553 B, with the priority of 21.10.1987, titled: "Process for preparation of isopolypeptides from diamino-monocarbonic acids and of drugs containing them, and a plant protection agent containing polyisolysine", further in the paper of Szókán et al.: "Structure Determination and Synthesis of Lysine Isopeptides Influencing on Cell Proliferation" (Biopolymers, J. Wiley & Sons, Inc. 42:305-318, 1997).

To a given representative of carrier molecules of general formula (**I/a**) within the new polycation bioconjugates of general formula (**I**), prepared according to the invention, practically any organic and/or inorganic molecule having functional groups appropriate for conjugation can be coupled as a suitably selected enhancer molecule, in accordance with the method shown by general formulae (**II**), (**III**), (**IV**), (**VI**), (**VII**), (**IX**), (**X**), (**XI**) and by schematic formulae (**V**), (**VIII**), (**IX/a**), (**X/a**), (**XI/a**). All these enhancer molecules can favourably be chosen – with a non-limiting manner – from the group of compounds listed hereinbelow:

- hormones and hormone antagonists of different kind (steroid, protein, peptide, etc.), and active fragments of peptide hormones, and derivatives thereof;
- saturated and unsaturated fatty acids, cholesterols, phospholipides (phosphoglycerides, sphingomyelins, etc.), and derivatives thereof;
- nucleic acids/antisense nucleotides;
- monosaccharides, oligosaccharides, and polysaccharides, and derivatives thereof;
- vitamines, and their derivatives;
- known antitumor drugs and active substances, and derivatives thereof;
- amino acids, oligopeptides, polypeptides, further glycoproteins and lipoproteins, their fragments, and derivatives thereof.

The new polycation bioconjugates of general formula (**I**) prepared according to the method described in the invention, contain carrier molecules of general formula (**I/a**), and a given representative of these carrier molecules is conjugated by chemical (covalent and/or ionic) bonds with enhancer molecules, which are suitably selected, according to the intended effect (for example antiproliferative, antimicrobial, gene delivery, improving of the quality of the diagnostic magnetic resonance imaging, etc.), and these enhancer molecules may either be identical ones or of (two or

more i.e. "x") different kind, and due to the applicability of multiple enhancer molecules in a given polycation bioconjugate of general formula (I), manifold direct and/or indirect effects can be obtained simultaneously. A few examples for the compounds that can favourably be applied to this purpose are listed hereinbelow.

Compounds comprising a part of the direct enhancer molecules – with non-limiting character:

- compounds having antiproliferative effects, for example: cytostatics used in the clinical practice, furthermore cytokines, which influence the division and differentiation of the cells (for example different growth factors, as well as antibodies produced against the receptors of these factors, interferons, etc.), furthermore peptides/proteins which inhibit the formation of new blood-vessels around the tumor cells (angiostatins, endostatins), furthermore nucleic acids/antisense oligonucleotides exerting antiproliferative effects on the malignantly transformed cells;
- compounds having antimicrobial effects, for example: antiviral, antibacterial, antimycotical, antiprotozooneal, etc. compounds, used in the clinical practice, furthermore nucleic acids/complexed antisense oligonucleotides, which inhibit the replication of the microbes;
- nucleic acids isolated or synthetized for the purpose of gene transfer, which are suitable for treating genetic diseases (for example cystic fibrosis);
- compounds improving the quality of the diagnostic magnetic resonance imaging, for example: paramagnetic metal ions and complexes containing metal ions of that kind, especially molecule complexes of gadolinium (Gd) ion (for example dimeglumine salt of Gd-diethylene-triamine-pentaacetic acid);
- compounds having immunomodulant effects (for example interleukins, tumor necrosis factors, etc.) which control a given function of the immune system; and
- numerous suitably selected compounds with other effects, not defined herein, that can be used with definite purposes, as enhancer molecules.

Compounds comprising a part of the indirect enhancer molecules, which develop or increase selectivity – with non-limiting character:

- in relation to the antiproliferative effects, for example: monoclonal antibodies having specific affinities to a surface antigen of a given tumor cell, as well as antibodies or any compound having affinity to those kind of receptors (for example transferrin receptor or folate receptor among the vitamins, etc.) which are present in a greater ratio on the surface of the tumor cells than of the normal (not malignantly transformed) cells;
- furthermore in the relation to the antiproliferative and any other effects, aimed at, any compound which has specific affinity to a certain receptor occurring exclusively on the surface of a given normal cell only (this receptor does not exsist as a result of a pathological process), namely for example the asialoglycoprotein cell surface receptors of the hepatic cells (to which specifically links the terminal galactose of the macromulecules), or any other compound coupling receptors, which are present in greater ratio on the surface of a given target cells;
- in general compounds which may link to a given target cell (for example microbes or infected cells with microbes, etc.) to achieve indirect enhancer effect.

Compounds comprising a part of the direct and simultaneously indirect enhancer molecules, with non-limiting character:

- in relation to the antiproliferative effects, for example: hormones, hormone antagonists and derivatives thereof, especially from among the polypeptide hormones the humane choriogonadotropine hormone, which having antiproliferative effects, furthermore antibodies produced against receptors of growth factors of different kind, which are present in greater ratio on the surface of a given tumor cell than on other cells, and simultaneously exert

antiproliferative effects towards given malignantly transfomed cells, furthermore immunotoxines, which are produced against a given tumor cell;

- in relation to the antimicrobial effects, for example: neutralizing antibodies which are produced against a given microbes (for examle viruses, bacteriums, funguses, etc.), furthermore immunotoxines, which are produced against a given microbe.

New polycation bioconjugates of general formula (**I**) prepared according to the invention contain carrier molecules of general formula (**I/a**), and a given representative of these carrier molecules is conjugated with enhancer molecules – which may either be identical ones or of (two or more i.e. "**x**") different kind – that are suitably selected according to the above mentioned examples, and the conjugation of these enhancer molecules are symbolized by $[Ex_i]_{p1}$ and/or $[...\text{-}Ex_{ek}]_{p3}$ which indicates that the molecules are coupled by covalent bonds, furthermore $[(\text{-})Ax_s]_t$ having anionic character and/or $[(+)Kx_u]_z$ having cationic character indicate the molecules that are coupled by ionic bonds.

New polycation bioconjugates of general formula (**I**) prepared according to the invention contain carrier molecules of general formula (**I/a**), and a given representative of these carrier molecules is conjugated with enhancer molecules – which may either be identical ones or of (two or more i.e. "**x**") different kind – that are suitably selected according to the above mentioned examples, and the enhancer molecules can be conjugated directly and/or indirectly through connecting molecules, and the latter can couple enhancer molecules covalently or ionically, symbolized by $[Cx_{ck}\text{-}...]_{p3}$ of the covalent ones, and by $[(\text{-})Cx_j]_{p2}$ of the ionic ones, respectively, and these connecting molecules may suitably be chosen – with non-limiting character – from dicarbonic acids, tricarbonic acids, carbohydrates, or amino acids, or peptide chain elongators.

New polycation bioconjugates of general formula (**I**) prepared according to the invention contain carrier molecules of general formula (**I/a**), and a given representative of these carrier molecules is conjugated with enhancer molecules – which may either be identical ones or of (two or more i.e. "**x**") different kind – that are suitably selected according to the above mentioned examples, and the conjugation of the enhancer molecules by covalent and/or ionic chemical bonds takes place directly and/or indirectly, in a determined ratio, preferably to reach a saturation of 10 to 100 %.

Preferred representatives of carrier molecules of general formula (**I/a**) within the new polycation bioconjugates of general formula (**I**), according to the invention include those 60 – 120 membered, non-racemic polyiso-L-lysines, i.e. poly(ε)-L-lysine-hydrogen-bromides which themselves possess certain antiproliferative and antiviral effects, as it is disclosed in the patent specification HU 202553 B, of the Hungarian priority of October 21, 1987.

Subject of the invention is the recognition that each of the new polycation bioconjugates of general formula (**I**), prepared according to the method described in the present invention, contains carrier molecules of general formula (**I/a**), and these carrier molecules (which themselves possess certain antiproliferative effects) are conjugated by chemical bonds with compounds having antiproliferative effects (some compounds, suitably selected to this purpose are listed above among the direct enhancer molecules), and the bioconjugates so obtained, are being successfully applicable for the treatment of malignancies, developing in mammal organisms (further: tumors), *in se*, or combined with known tumor-inhibiting methods, accepted in the clinical practice.

The additional enhancer molecules, resulting in developing an appropriately chosen selectivity, that have particularly been disclosed hereinabove (among the indirect enhancer molecules), linked to the bioconjugates prepared according to the invention, are increasing the concentration

of active substances in the tumors, whereby the unwanted side-effects can be diminished, and thus the effectiveness of the treatment may further be increased.

Conjugates similar to those new polycation bioconjugates of antiproliferative effect, according to the present invention, have already been prepared earlier. Papers have also been published about them, from among which we would refer to some as follows hereinbelow:

- Bogdanov-A.A Jr., Martin-C., Bogdanova-A.V. et al.: An adduct of cis-diammine-dichloroplatinum(II) and poly(ethylene glycol)poly(L-lysine)-succinate: synthesis and cytotoxic properties; *Bioconjug-Chem. 1996 Jan-Feb; 7(1): 144-9.*
- Di-Stefano-G., Busi-C., Derenzini-M. et al.: Conjugation of 5-fluoro-2'-deoxyuridine with lactosaminated poly-l-lysine to reduce extrahepatic toxicity in the treatment of hepatocarcinomas; *Ital-J-Gastroenterol-Hepatol. 1998 Apr; 30(2): 173-7.*
- Paprocka-M., Boratynski-J., Dus-D. et al.: Conjugation of the monoclonal antibody 17-1A with the nitroacridine compound C921 with the poly-L-lysine as an intermediate agent; *Arch-Immunol-Ther-Exp-Warsz. 1997; 45(4): 343-9.*
- Salazar-A.M., Levy-H.B., Ondra-S. et al.: Long-term treatment of malignant gliomas with intramuscularly administered polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose: an open pilot study; *Neurosurgery. 1996 Jun; 38(6): 1096-103; discussion 1103-4.*

Another recognition contained in the present invention is the feature of the carrier molecules of general formula (I/a), in the new polycation bioconjugates of general formula (I), that as being polycations, they are appropriate for transporting of, as well as introducing to the target cells the suitably selected nucleic acids of polyanionic character, as enhancer molecules, bound to them by ionic bonds, i.e. for gene transfer, using the effect that by conjugating further enhancer molecules by covalent bonds – details of which see hereinabove – resulting in developing an appropriately chosen selectivity, the new polycation bioconjugates are being linked selectively to the target cells, or in essentially higher ratio to them than to cells of other type.

Conjugates similar to those new polycation bioconjugates, according to the present invention, capable of gene transfer, have already been prepared earlier. Papers have been published about them in the scientific literature, from among which we would refer to some publications – as examples – hereinbelow:

- Erbacher-P., Roche-A.C., Monsigny-M., Midoux-P.: The reduction of the positive charges of polylysine by partial gluconoylation increases the transfection efficiency of polylysine/DNA complexes; *Biochim. Biophys.Acta. 1997 Feb 21; 1324(1): 27-36.*
- Ferkol-T., Perales-J.C., Mularo-F., Hanson-R.W.: Receptor-mediated gene transfer into macrophages; *Proc-Natl-Acad-Sci-USA. 1996 Jan 9; 93(1): 101-5.*
- Kollen-W., Erbacher-P., Midoux-P. et al.: Glycosylated polylysines. Nonviral vectors for gene transfer into cystic fibrosis airway epithelial cells; *Chest. 1997 Jun; 111(6 Suppl): 95S-96S.*
- Liang-W.W., Shi-X., Deshpande-D. et al.: Oligonucleotide targeting to alveolar macrophages by mannose receptor-mediated endocytosis; *Biochim-Biophys-Acta. 1996 Mar 13; 1279(2): 227-34.*
- Schneider-H., Huse-K., Birkenmeier-G. et al.: Gene transfer mediated by alpha2-macroglobulin; *Nucleic-Acids-Res. 1996 Oct 1; 24(19): 3873-4.*
- Schwarzenberger-P., Spence-S.E., Gooya-J.M. et al.: Targeted gene transfer to human hematopoietic progenitor cell lines through the c-kit receptor; *Blood. 1996 Jan 15; 87(2): 472-8.*

- Sosnowski-B.A., Gonzalez-A.M., Chandler-L.A. et al.: Targeting DNA to cells with basic fibroblast growth factor (FGF2); *J-Biol-Chem. 1996 Dec 27; 271(52): 33647-53.*
- Stewart-A.J., Pichon-C., Meunier-L. et al.: Enhanced biological activity of antisense oligonucleotides complexed with glycosylated poly-L-lysine; *Mol-Pharmacol. 1996 Dec; 50(6): 1487-94.*

Furthermore the new polycation bioconjugates which are suitable for gene transfer in the case of further conjugation with compounds having antiproliferative effects (the suitably selected compounds, detailed among the direct enhancer molecules) are suitable for more effective treatment of the tumors. Scientific reprints have been also published about these, from among which we refer to some as follows hereinbelow:

- Cristiano-R.J., Roth-J.A.: Epidermal growth factor mediated DNA delivery into lung cancer cells via the epidermal growth factor receptor; *Cancer-Gene-Ther. 1996 Jan-Feb; 3(1): 4-10.*
- Foster-B.J., Kern-J.A.: HER2-targeted gene transfer; *Hum-Gene-Ther. 1997 Apr 10; 8(6): 719-27.*
- Ginobbi-P., Geiser-T.A., Ombres-D., Citro-G.: Folic acid-polylysine carrier improves efficacy of c-myc antisense oligodeoxynucleotides on human melanoma (M14) cells; *Anticancer-Res. 1997 Jan-Feb; 17(1A): 29-35.*
- Nguyen-D.M., Wiehle-S.A., Roth-J.A., Cristiano-R.J.: Gene delivery into malignant cells in vivo by a conjugated adenovirus/DNA complex; *Cancer-Gene-Ther. 1997 May-Jun; 4(3): 183-90.*
- Schachtschabel-U., Pavlinkova-G., Lou-D., Kohler-H.: Antibody-mediated gene delivery for B-cell lymphoma in vitro; *Cancer-Gene-Ther. 1996 Nov-Dec; 3(6): 365-72.*
- Shimizu-N., Chen-J., Gamou-S., Takayanagi-A.: Immunogene approach toward cancer therapy using erythrocyte growth factor receptor-mediated gene delivery; *Cancer-Gene-Ther. 1996 Mar-Apr; 3(2): 113-20.*
- Watanabe-N., Sato-Y., Yamauchi-N., Niitsu-Y.: Gene delivery into human cancer cells via transferrin receptor; *Nippon-Rinsho. 1998 Mar; 56(3): 724-30.*

Another recognition also belongs to the subject of the present invention, namely that each of the new polycation bioconjugates of general formula (**I**) prepared according to the method described in the invention, contains carrier molecules of general formula (**I/a**) and these carrier molecules (which possess certain antiviral effects themselves) are conjugated, by chemical bond, with suitably selected compounds having antiviral effects, as direct enhancer molecules, and due to this, increase the antiviral effect of the new polycation bioconjugates.

New polycation bioconjugates which are produced in the way described in the paragraph before, conjugated furthermore, by chemical bond, with suitably selected compounds which develop or increase selectivity to the target cells which are infected by the virus (the suitably selected compounds detailed among the indirect enhancer molecules) are suitable to increase the relative concentration of the new antiviral character polycation bioconjugates in the cells which are infected by the virus and due to it the efficacy of the treatment will be increased and side-effects greatly diminished. Conjugates similar to those new polycation bioconjugates of antiviral effect, according to the present invention, have already been prepared earlier. Papers have also been published about them, from among which we would refer to some as follows hereinbelow:

- Di-Stefano-G., Colonna-F.P., Bongini-A. et al.: Ribavirin conjugated with lactosaminated poly-L-lysine: selective delivery to the liver and increased antiviral activity in mice with viral hepatitis; *Biochem-Pharmacol. 1997 Aug 1; 54(3): 357-63.*

- Fiume-L., Di-Stefano-G., Busi-C. et al.: Liver targeting of antiviral nucleoside analogues through the asialoglycoprotein receptor; *J-Viral-Hepat. 1997; 4(6): 363-70.*
- Fiume-L; Di-Stefano-G; Busi-C. et al.: Hepatotropic conjugate of adenine arabinoside monophosphate with lactosaminated poly-L-lysine. Synthesis of the carrier and pharmacological properties of the conjugate; *J-Hepatol. 1997 Feb; 26(2): 253-9.*
- Nakazono-K., Ito-Y., Wu-C.H., Wu-G.Y.: Inhibition of hepatitis B virus replication by targeted pretreatment of complexed antisense DNA in vitro; *Hepatology. 1996 Jun; 23(6): 1297-303.*

Another recognition also belongs to the subject of the present invention, namely that each of the new polycation bioconjugates of general formula (**I**) prepared according to the method described in the invention, contains carrier molecules of general formula (**I/a**) and these carrier molecules are conjugated, by chemical bond, with compounds having different kind of antimicrobial effects (the suitably selected compounds, detailed among the direct enhancer molecules), and due to this, the new polycation bioconjugates significantly increase the antimicrobial effects surpassing the antiviral character, which was described in the three previous paragraph.

New polycation bioconjugates which are produced in the way described in the paragraph before, conjugated furthermore, by chemical bond, with compounds which develop or increase selectivity to the target cells which are infected by different kind of microbas (the suitably selected compounds, detailed among the indirect enhancer molecules) are suitable to increase the relative concentration of the antimicrobial character polycation bioconjugates in the cells which are infected by the given microbas and due to this the efficacy of the treatment will be increased and side-effects greatly diminished.

Another recognition also belongs to the subject of the present invention, namely that each of the new polycation bioconjugates of general formula (**I**) prepared according to the method described in the invention, contains carrier molecules of general formula (**I/a**) and these carrier molecules are conjugated, by chemical bond, with macromolecular paramagnetic contrast agents for example the gadolinium molecule complexes, and derivatives thereof, (the suitably selected compounds, detailed among the direct enhancer molecules), and due to this, the new polycation bioconjugates significantly improve the quality of the diagnostic magnetic resonance imaging, by increasing the contrast between the different kind of organs, tissues, as well as the different pathological alterations, for example tumors.

New polycation bioconjugates which are produced in the way described in the paragraph before, conjugated furthermore, by chemical bond, with compounds which develop or increase selectivity to the different kind of target organs or different pathological alterations (the suitably selected compounds, detailed among the indirect enhancer molecules), are suitable to increase significantly the relative concentration of the paramagnetic contrast character polycation bioconjugates in the different kind of organs, tissue, as well as, the different pathological alterations, and due to this further improve the quality of the magnetic resonace imaging.

Conjugates similar to those new polycation bioconjugates of paramagnetic character, according to the present invention, have already been prepared earlier. Papers have also been published about them, from among which we would refer to some as follows hereinbelow:

- Opsahl-L.R., Uzgiris-E.E., Vera-D.R.: Tumor imaging with a macromolecular paramagnetic contrast agent:gadopentetate dimeglumine-polylysine; *Acad-Radiol. 1995 Sep; 2(9): 762-7.*

- Su-M.Y., Muhler-A., Lao-X., Nalcioglu-O.: Tumor characterization with dynamic contrast-enhanced MRI using MR contrast agents of various molecular weights; *Magn-Reson-Med. 1998 Feb; 39(2): 259-69.*
- Vera-D.R., Buonocore-M.H., Wisner-E.R. et al.: A molecular receptor-binding contrast agent for magnetic resonance imaging of the liver; *Acad-Radiol. 1995 Jun; 2(6): 497-506.*
- Vogl-T.J., Hoffmann-Y., Juergens-M. et al.: Experimentelle Evaluierung der kontrastmittelverstärkten, hochauflösenden MR-Angiographie am Tiermodell. Gd-DTPA gegenüber Gd-DTPA-Polylysin; *Radiologie. 1996 Mar; 36(3): 254-62.*

Another recognition also belongs to the subject of the present invention, namely that each of the new polycation bioconjugates of general formula (**I**) prepared according to the method described in the invention, contains carrier molecules of general formula (**I/a**) and these carrier molecules as polycations make suitable the polycation bioconjugates to get into the mammal organism, via transdermal transport by iontophoresis.

The new polycation bioconjugates which are trasported through the skin, exert their effects mainly in the different strata of the skin, and in the subcutan tissues, at the area of the iontophoresis, and certain amount of them act systemic. These kind of actions depend on the molecular size, the physico-chemical character and the type of the suitably selected direct and/or indirect enhancer molecule of the polycation bioconjugates, as well as, on the nature of the applied electric field.

The concentration of the new polycation bioconjugates, which are prepared according to the therapeutical aims (for example antiproliferative or antiviral effects, etc.) and which contain the suitably selected and above detailed direct and/or indirect enhancer molecules, increases at the place of the transdermal application, and due to this the local efficacy of the treatment will be greatly increased and side-effects diminished. If the aim is to achieve a systemic effect via transdermal application, the advantage will manifest in a constant, non invasiv administration of the polycation bioconjugates, wich avoid the gastro-intestinal system. Conjugates similar to those new polycation bioconjugates capable of transdermal application, according to the present invention, have already been prepared earlier. Papers have also been published about them, from among which we would refer to some as follows hereinbelow:

- Turner-N.G., Ferry-L., Price-M. et al.: Iontophoresis of poly-L-lysines: the role of molecular weight?; *Pharm-Res. 1997 Oct; 14(10): 1322-31.*
- Vanbever-R., Prausnitz-MR., Preat-V.: Macromolecules as novel transdermal transport enhancers for skin electroporation; *Pharm-Res. 1997 May; 14(5): 638-44.*

Another recognition also belongs to the subject of the present invention, namely that each of the new polycation bioconjugates of general formula (**I**) prepared according to the method described in the invention, contains carrier molecules of general formula (**I/a**), and these carrier molecules are conjugated with above detailed, direct and/or indirect enhancer molecules, which are suitably selected according to a given therapeutical purpose (for example antiproliferative or antiviral effects or gene therapy, etc.), and these new polycation bioconjugates are to be placed into cationic liposomes, and due to this the efficacy of the treatments will be increased and the side-effects greatly diminished. Conjugates similar to those new polycation bioconjugates which are suitable to be placed into cationic liposomes, according to the present invention, have already been prepared earlier. Papers have also been published about them, from among which we would refer to some as follows hereinbelow:

- Gao-X., Huang-L: Potentiation of cationic liposome-mediated gene delivery by polycations; *Biochemistry. 1996 Jan 23; 35(3): 1027-36.*
- Lee-R.J., Huang-L.: Folate-targeted, anionic liposome-entrapped polylysine-condensed DNA for tumor cell-specific gene transfer; *J-Biol-Chem. 1996 Apr 5; 271(14): 8481-7.*
- Mack-K.D., Walzem-R.L., Lehmann-Bruinsma-K. et al.: Polylysine enhances cationic liposome-mediated transfection of the hepatoblastoma cell line Hep G2; *Biotechnol-Appl-Biochem. 1996 Jun; 23 (Pt 3): 217-20.*
- Saldeen-J., Curiel-D.T., Eizirik-D.L. et al.: Efficient gene transfer to dispersed human pancreatic islet cells in vitro using adenovirus-polylysine/DNA complexes or polycationic liposomes; *Diabetes. 1996 Sep; 45(9): 1197-203.*
- Vitiello-L., Chonn-A., Wasserman-J.D. et al.: Condensation of plasmid DNA with polylysine improves liposome-mediated gene transfer into established and primary muscle cells; *Gene-Ther. 1996 May; 3(5): 396-404.*
- Zelphati-O., Szoka-F.C Jr.: Mechanism of oligonucleotide release from cationic liposomes; *Proc-Natl-Acad-Sci-U-S-A. 1996 Oct 15; 93(21): 11493-8*

The biologically effective conjugates which have been described in the scientific reviewes of medicine, and cited above, contain carrier molecules, which are build up from diamino-monocarbonic acid monomers, namely lysines, that are coupled to each other by peptide bonds through amino groups in the α-positions, therefore as a result of their synthesis, poly-(α)-L-lysine is formed, and these facts support anyway the recognitions of the present invention, indirectly.

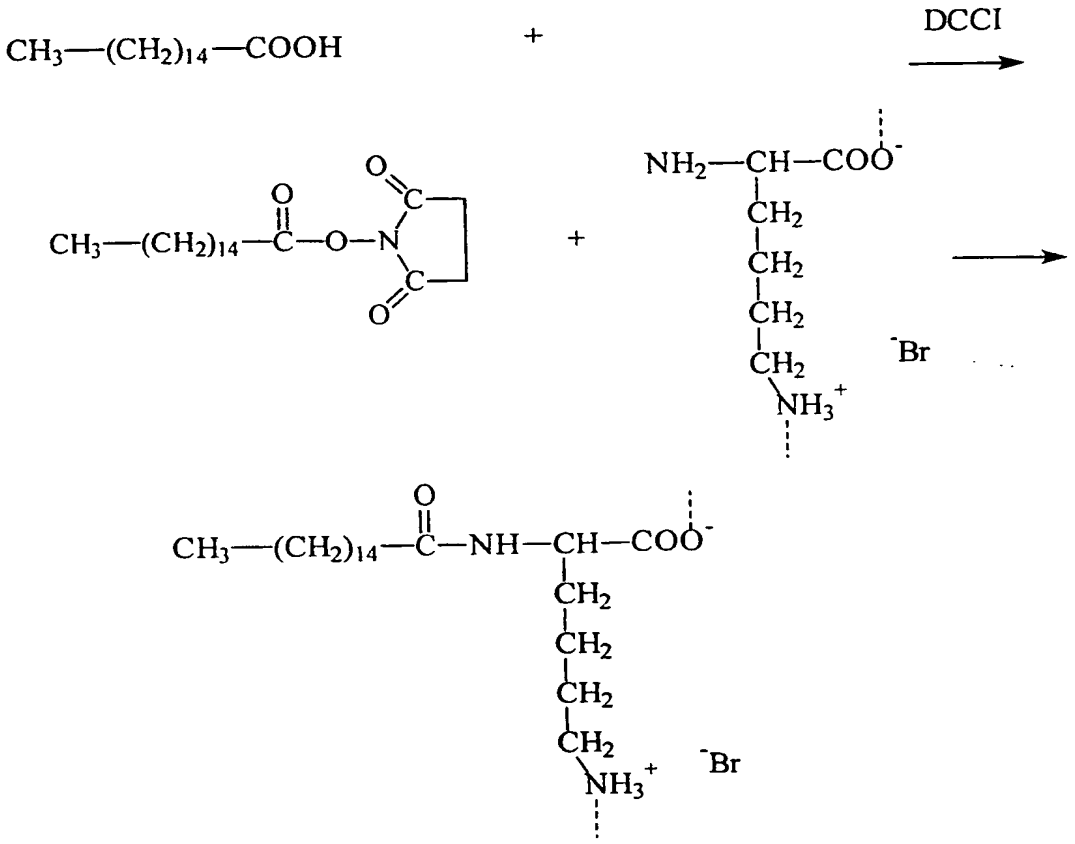
On the basis of all above aspects, the novelty of the invention is comprising in that each of the polycation bioconjugates of general formula (**I**), prepared according to the invention, contains carrier molecules of general formula (**I/a**), and these carrier molecules are build up fom diamino-monocarbonic acid monomers, which are coupled by peptide bonds formed via the amino groups in the (β-, γ-, δ-, ε-..,etc.) positions, corresponding to the value of "m", and not through amino groups in the α-position, and therefore, as a result of the synthesis β-, γ-, δ-, ε-.., etc. polypeptides are forming, which are structurally entirely different from those polypeptides that have been described in the cited scientific reviews. The biological behaviour of the new polycation bioconjugates of general formula (**I**) will therefore be altered. For instance, they are more resistant against proteolytic enzymes, further the carrier molecules of general formula (**I/a**) themselves possess certain antiproliferative, antiviral activity, and as a consequence, the biological effectiveness of the new polycation bioconjugates of general formula (**I**) is being modified favourably. The new polycation bioconjugates of general formula (**I**) according to the invention are being formulated as pharmaceutical preparates that are applicable perorally, parenterally, or transdermally, for systemic or topical use.

Preferred representatives of the new polycation bioconjugates of general formula (**I**) according to the invention are those bioconjugates, in which the carrier molecules of general formula (**I/a**) are poly-(ε)-L-lysines, whereas the preparation of some representatives thereof is illustrated by the following examples hereinbelow:

Example 1

Preparation of palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide

The reaction scheme:



Formula of palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide - according to the general formula **(II)** of the new polycation bioconjugates of the invention - is:

$$H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH(-NH\text{-}[Ex_i]_{p1})\text{-}CO]_rOH \qquad \textbf{(II)}$$

wherein:
"**m**" = 3, "**r**" = 99 ± 2,
"**E**" = $CH_3—(CH_2)_{14}—CO—$,
"**i**" = 1; i.e. only one single kind of enhancer molecules is being linked by chemical bond to the carrier molecule, and
"$\mathbf{p_1}$" = 12 ± 2 %.

a) Poly-(ε)-L-lysine-hydrogen-bromide (mean M_w = 12700±200, specific optical rotation = +32,4°, degree of polymerisation: R = 99 ± 2), as a carrier molecule according to the invention, of the general formula (I/a), was synthetized following the method described in Example 1 i) of the Hungarian patent specification HU 202553 B.
b) preparing of palmitoyl-N-hydroxy-succinimide needed to the palmitoylation reaction: 1,28 g (5 mmole) of palmitoic acid and 0,58 g (5 mmole) N-hydroxy-succinimide were dissolved in 12 ml of abs. tetrahydrofurane, and to this solution 1,03 g (5 mmole) of dicyclohexyl-

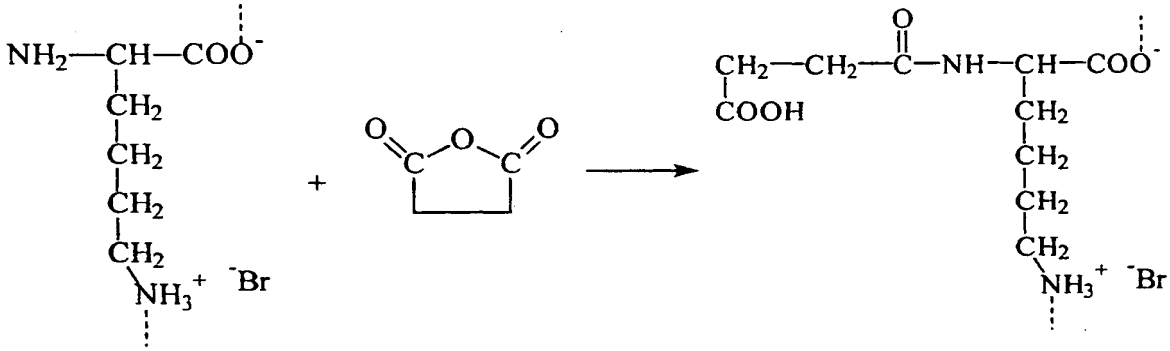
carbodiimide was added, then the mixture was stirred for 4 hours at 0°C, subsequently left standing at +4°C for 12 hours and the precipitated dicyclohexyl-carbamide that formed during the process was glass-filtered in vacuo and was washed three times with tetrahydrofurane, the clear solution so obtained was concentrated to dryness, the solid residue was dissolved in 100 ml of ethyl acetate, and the latter was washed three times with 5% sodium bicarbonate solution, then three times with water, which resulted in 1,08 g of palmitoyl-N-hydroxy-succinimide end product in white, crystalline form. Its purity was checked by TLC and IR.

c) 250 mg of poly(ε)-L-lysine-hydrogen-bromide prepared according to the method described in Example 1a) was dissolved in 1,5 ml of water, pH of the solution was adjusted to 8, by adding 1N NaOH, under vigorous stirring, then the solution was cleared up by adding 1,5 ml of tetrahydrofurane and 0,5 ml of dimethyl formamide, and after addition of 50 mg of sodium bicarbonate under vigorous stirring, 28 mg of palmitoyl-N-hydroxy-succinimide, freshly prepared according to Example 1b), and dissolved in 0,2 ml of 6:1 tetrahydrofurane/dimethyl formamide mixture, was added to the reaction mixture, followed by 4 hours of vigorous stirring, while the pH was kept on 8, by dropping 5N sodium hydroxide into the solution, and it was left standing for 12 hours at +4°C, afterwards. Following this 0,1 ml of azeotropic hydrogen bromide solution was added dropwise, then the reaction mixture was poured into an excess (20 ml) of tetrahydrofurane, the precipitate was washed with tetrahydrofurane three-four times, until it became powdery, then the latter was washed two times with diethylether and dried subsequently; the palmitoyl-poly(ε)-L-lysine-hydrogen-bromide end product was obtained this way. The yield was 245 mg. Free amino groups of the product were checked by trinitrobenzene sulfonic acid analysis, according to which the degree of substitution was 12±2%. By increasing the amount of palmitoyl-N-hydroxy-succinimide reactant, the degree of substitution also increased, and reached the necessary % level.

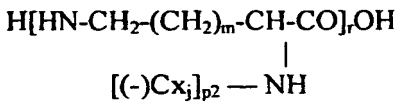
Example 2

Preparation of hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide

The reaction scheme:



Formula of hydrobromide salt of hemisuccinyl-poly-(ε)-L-lysine - according to the general formula (**III**) of the new compounds of the invention - is:



(III)

wherein:

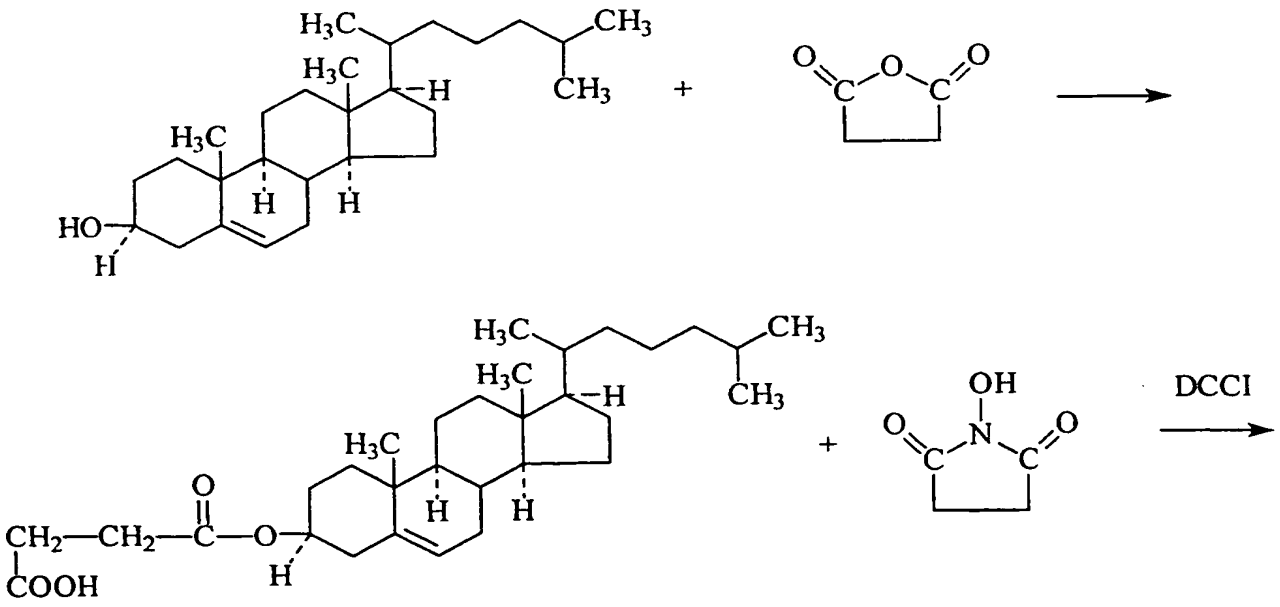
"**m**" = 3, "**r**" = 99 ± 2;
"(-)C" = —OC—CH_2—CH_2—COOH;
"**j**" = 1; i.e. only one single kind of connecting molecules is being linked by covalent bonds to the carrier molecule, and
"p_2" = 20 ± 2 %.

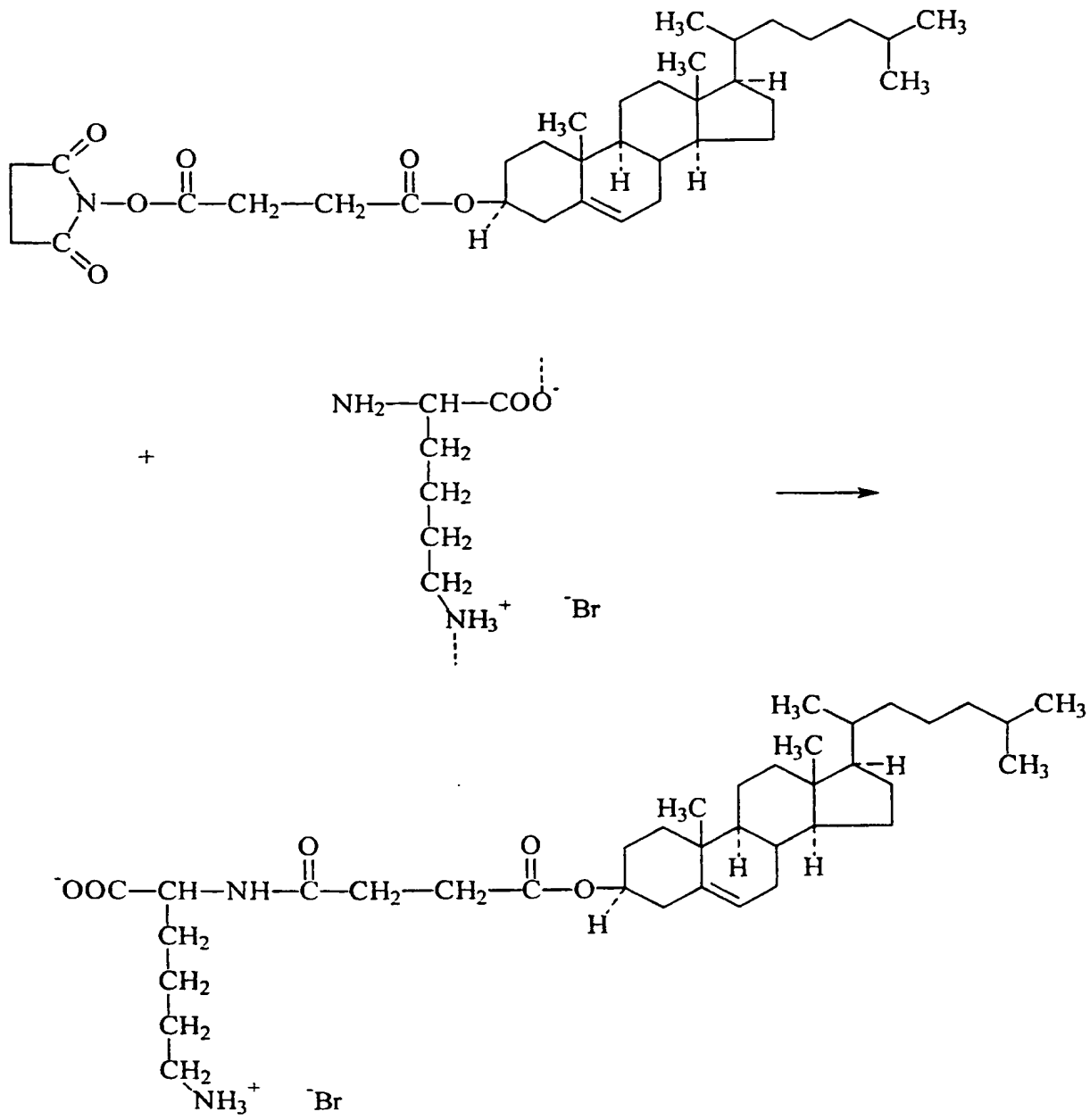
a) Poly-(ε)-L-lysine-hydrogen-bromide, as being a carrier molecule of general formula (**I/a**) of the invention, was synthetized according to Example 1a) of the present patent application.
b) 60 mg of poly-(ε)-L-lysine-hydrogen-bromide prepared according to Example 1a) was dissolved in 2 ml of water, and the pH of the solution was adjusted to 8, by adding 1N sodium hydroxyde, under vigorous stirring, then 30 mg of freshly prepared succinic anhydride was added in portions to the solution, in about 40 minutes time, whereas keeping the pH on 8 was accomplished by dropping 5N NaOH, followed by stirring for 40 minutes, additionally, and at the end of the reaction pH of the mixture was lowered to 4,5 by 6N hydrochloric acid, then it was filled into a dialysing bag and has been dialysed against water for 48 hours, subsequently at +4°C temperature, by changing four times the water, the hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide salt end product was isolated by freeze-drying. The yield was 46 mg. Free amino groups of the product were checked by trinitro benzene sulfonic acid analysis, according to which the degree of substitution was 20%. By increasing the amount of succinic acid, degree of substitution has also been increased, and reached a proper, necessary % level.

Example 3

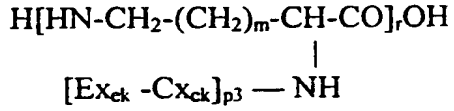
Preparation of cholesterol-hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide

The reaction scheme:





Formula of hydrobromide salt of cholesterol-hemisuccinyl-poly-(ε)-L-lysine – according to the general formula (IV) of the new polycation bioconjugates of the invention – is:



(IV)

wherein:

"**m**" = 3, "**r**" = 99 ± 2;
"**C**" = —OC—CH_2—CH_2—CO—;

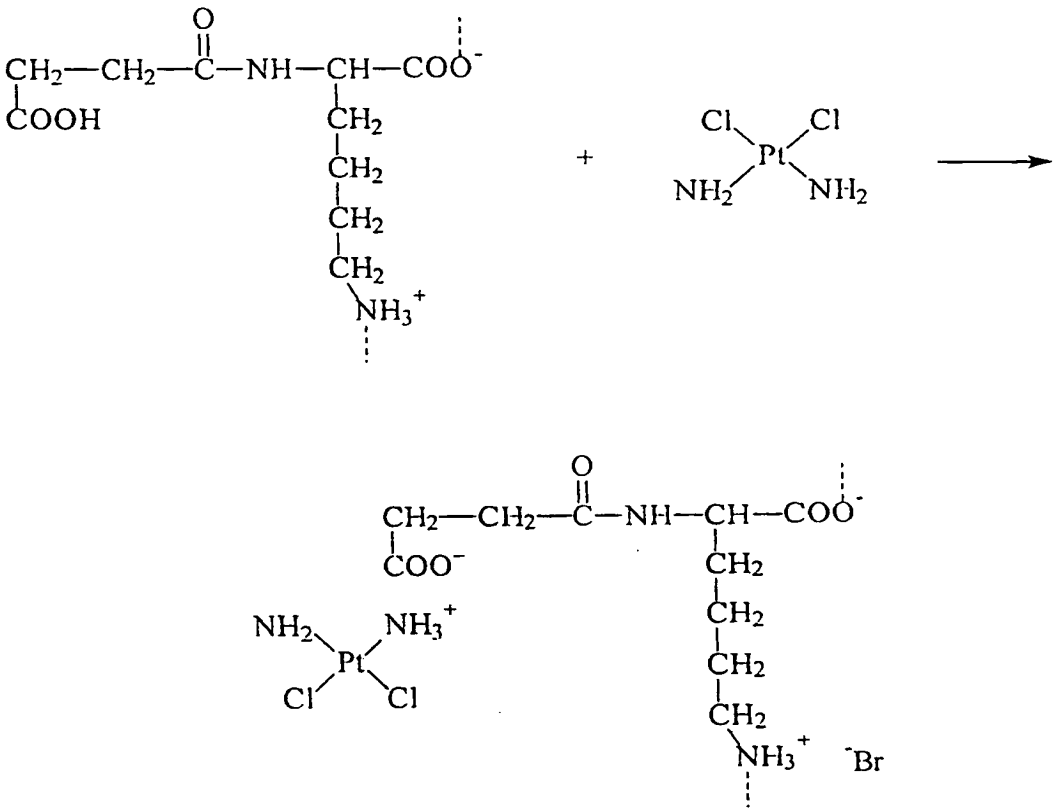
"**E**" = cholesterol;
"**ek**" = "**ck**" = 1; i.e. only one single kind of enhancer and of connecting molecules is being linked by covalent bonds to the carrier molecule, and
"p_3" = 15 ± 2 %.

a) Poly-(ε)-L-lysine-hydrogen-bromide, as being a carrier molecule of general formula (**I/a**) of the invention, was synthetized according to Example 1a) of the present patent application.
b) preparation of cholesterol-hemisuccinate-N-hydroxy-succinimide ester needed to the conjugation: 0,98 g (2 mmole) of commercially available (eg. from Sigma) cholesterol-hemisuccinate and 0,23 g (2 mmole) of N-hydroxy-succinimide were dissolved in 10 ml of abs. tetrahydrofurane, 0,41 g (2 mmole) of dicyclohexyl-carbodiimide was then added, and the mixture was stirred at 0°C, for 4 hours, then left standing for 12 hours, and the thus formed precipitate dicyclohexyl-carbamide was glass-filtered in vacuo, washed three times with tetrahydrofurane, and the clear solution so obtained was concentrated to dryness, the solid residue was dissolved in 50 ml of ethyl acetate, and the latter was washed three times with 5% sodium bicarbonate solution, then three times with water. Subsequently it was dried with adding sodium sulfate sicc., followed by washing with ethyl acetate and concentration of the solution. The cholesterol-hemisuccinate-N-hydroxy-succinimide ester end product was obtained in the form of white, crystalline material. The yield was 0,73 g. Purity was checked by TLC and by IR.
c) 240 mg of poly-(ε)-L-lysine-hydrogen-bromide salt synthetized according to Example 3a) was dissolved in 4,8 ml of water and the pH of the solution was adjusted to 8, by 1N sodium hydroxyde, under vigorous stirring, and by adding 1,6 ml of tetrahydrofurane a clear solution was obtained, then 200 mg of sodium bicarbonate was poured into it under vigorous stirring, by adding of 50 mg cholesterol-hemisuccinate-N-hydroxy-succinimide ester, freshly prepared according to Example 3b), dissolved in 2,4 ml tetrahydrofurane, and the solution was kept strongly stirred at room temperature for 4 hours, while keeping the pH at a value of 8 by dropping 5N sodium hydroxyde into the solution. Subsequently it was left standing for 12 hours at +4 °C, then 0,3 ml of azeotropic hydrogen bromide was dropped in, and the reaction mixture was poured into an excess (30 ml) of tetrahydrofurane. The precipitate was washed thereafter with tetrahydrofurane three-to-four times, until it became powdery. The latter was washed with diethyl ether two times, dried, and the cholesterol-hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide salt end product was obtained this way with 275 mg yield. Free amino groups of the product were checked by trinitro benzene sulfonic acid analysis, according to which the degree of substitution was 15±2%. By increasing the amount of cholesterol-hemisuccinate-N-hydroxy-succinimide ester, the degree of substitution also increased, and reached a proper, necessary % level.

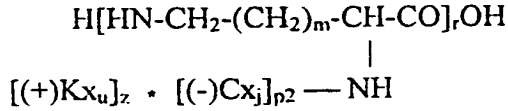
Example 4

Preparation of poly-(ε)-L-lysine-cisplatin-hydrogen-bromide

The reaction scheme:



Formula of poly-(ε)-L-lysine-cisplatin-hydrogen-bromide – according to the new polycation bioconjugates of general formula (**VII**) of the invention – is:



(VII)

wherein:

"**m**" = 3, "**r**" = 99 ± 2,
"(-)**C**" = —OC—CH_2—CH_2—COO^-,
"(+)**K**" = cisplatin,
"**u**" = "**j**" = 1; i.e. only one single kind of enhancer and of connecting molecules, respectively are being linked ionic bonds to the carrier molecule, and
"**u**" = "p_2" = 80 ± 2 %.

10 mg cisplatin (Platidiam®, manufacturer: Lachema, Czech Rep.) was dissolved in 3 ml of water and to this solution 23 mg of hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide salt of 80% succinylation, prepared according to Example 1 of the present patent application, was

added, the solution was left standing for 48 hours at +4 °C, then it was filled into a dialysing bag and has been dialysed against water for 48 hours at +4°C temperature, by changing the water four times, the poly-(ε)-L-lysine-cisplatin-hydrogen-bromide end product was subsequently isolated by freeze-drying. The yield was 15 mg. The Pt-content (i.e. the content of cisplatin) of the product was assayed by atomic absorption spectroscopy, according to which the Pt/Br mass ratio=16; the molar ratio=6,5; the Pt-content=2,7 mg/g; the amount of cisplatin in the poly-(ε)-L-lysine-cisplatin-hydrogen-bromide molecule has been proportional to the degree of succinylation of hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide.

BIOLOGICAL EXAMINATIONS

The tumor inhibitory effect of the polycation bioconjugates, which is a part of the subject of the invention, were studied, *in vitro*, on tumor cell cultures and *in vivo*, on transplantable rodent tumors. The *in vitro* inhibitory effect on cell proliferation and the *in vivo* tumor growth inhibitory effect of the different polycation bioconjugates, prepared according to the invention, was compared to the untreated control ones.

The biological experiments were carried out with the compounds, that were prepared according to the examples No. 1, 2, 3 and 4 of the invention, namely

- palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide,
- hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide,
- cholesterol-hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide,
- poly-(ε)-L-lysine-cisplatin-hydrogen-bromide.

In vitro experiments

In vitro experiments were carried out with palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide, hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide and poly-(ε)-L-lysine-cisplatin-hydrogen-bromide.

Cell lines used in the experiments:

- P388 mouse lymphocytic leukemia cell line, originated from Arthur D. Little Inc., Cambridge, Mass., USA, *in vitro* established in the National Institute of Oncology, Budapest, Hungary (Cancer Treat. Rep. 1986; 70: 279-284);
- MCF-7 humane breast cancer cell line, originated from American Type Culture Collection;
- PC3 humane prostate cancer cell line, originated from American Type Culture Collection;

Methods used in the *in vitro* experiments:

1./ Clonogenic assay:

MCF-7, or PC3 cells were plated into petri dishes, then in adequate medium and conditions, were incubated the cells with serial dilutions of the different polycation bioconjugates, prepared according to the invention. The colonies, consisting minimum 30 cells were then counted and the obtained values of three parallel petri dishes were averaged. Relative cloning efficiency was calculated by taking the control values as 100 per cent. (Cancer Detection and Prevetion, 20(2): 146-152, 1996).

2./ Inhibition of proliferation:

- Sulforodamine B (SRB) assay: approximately one to two thousand cells were put into each well of the special, small plastic tray (micro-well-plates). The cells were incubated in

adequate medium and conditions and after adhering to the surface, were treated with serial dilutions of the different polycation bioconjugates, prepared according to the invention. At the end of the experiment the cells were fixed, stained with SRB, and the optical density, which is in direct proportion to the cell proliferation, was read in a CLS 962 ELISA microplate reader. Relative inhibition of proliferation was calculated by taking the control values as 100 per cent. (Cancer Detection and Prevetion, 20(2): 146-152, 1996).

- Cell counting method, with Neubauer-type chamber: in suspension cultures of exponentially growing cells, which were in adequate medium and conditions, were treated with serial dilutions of the different polycation bioconjugates, prepared according to the invention, and 24- and 48 hours following the treatment the cells were stained with tripan-blue, the treated and control cells were counted in a modified Neubauer-type hemocytometer chamber. Relative inhibition of proliferation was calculated by taking the control values as 100 per cent.

In vivo experiments

The *in vivo* experiments were carried out with palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide and cholesterol-hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide as a monotherapy, and together with Cytoxan® (Bristol-Myers) as combination therapy, and the compounds were administrated in schedules of single or repeated dose therapy, and they were applied intravenously or intraperitoneally.

The tumor growth inhibitory effect of the polycation bioconjugates was studied on the following transpalatable rodent tumors:

- P-388 lymphoid leukemia cells, originated from Cambridge, Mass., USA, were transplanted *i.p.* and *s.c.* into animals: BDF1 inbred male mice, weighing 22-24 g, specified pathogen free (SPF) breedings;
- S-180 sarcoma, originated from Chester Beatty I., London, were transplanted *s.c.* into animals: BDF1 inbred male mice, weighing 22-24 g, specified pathogen free (SPF) breedings.

Results of the biological experiments

In vitro experiments

On tumor cell cultures, *in vitro*, the palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide, cholesterol-hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide and poly-(ε)-L-lysine-cisplatin-hydrogen-bromide exert a dose dependent inhibition on the colony formation and the cell proliferation, the most effective inhibition have been shown by the palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide, and poly-(ε)-L-lysine-cisplatin-hydrogen-bromide.

In vivo experiments

On the basis of mean tumor volumes and tumor growth curves we observed a significant inhibitory effect of the palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide and cholesterol-hemisuccinyl-poly-(ε)-L-lysine-hydrogen-bromide on transplantable rodent tumors, furthermore the combined treatment with palmitoyl-poly-(ε)-L-lysine-hydrogen-bromide plus Cytoxan® significantly inhibited the tumor growth and significantly increased the life span of the experimental animals inoculated with tumor, compered to the untreated control, as well as to the animals which were treated only with Cytoxan®.

What we claim is:

1) Polycation bioconjugates c h a r a c t e r i z e d in that each of them contains isopolypeptide polycation carrier molecules having free α-amino groups, and these carrier molecules are conjugated by chemical bonds with suitably selected molecules which may either be identical ones or of (two or more i.e. "**x**") different kind, bearing functional groups appropriate for conjugation, and the polycation bioconjugates synthetized this way can be described by the general formula (**I**):

$$\begin{array}{l} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ \qquad\qquad\qquad\qquad\qquad\quad | \\ [(i)Mx] \cdot [(k)Mx] \text{ —— } NH \end{array} \qquad \textbf{(I)}$$

wherein:

"**r**" is a mean value between 20 and 400;
"**m**" = 0, 1, 2, 3, ... k;
"[(k)**M**x]" designates enhancer molecules and/or connecting molecules conjugated by covalent (= **k**) bonds to the isopolypeptide polycation carrier molecule, and
"[(i)**M**x]" designates enhancer molecules conjugated by ionic (= **i**) bonds to the isopolypeptide polycation carrier molecule, whereas the said enhancer molecules and connecting molecules having appropriate functional groups for conjugation may either be identical ones or of (two or more i.e. "**x**") different kind, and the enhancer molecules can be conjugated:

- directly and/or
- indirectly through a connecting molecule,

and further the joint occurrence of [(k)**M**x] and [(i)**M**x] within the same polycation bioconjugate is symbolized by [(k/i)**M**x].

2) Polycation bioconjugates of general formula (**I**), prepared according to Claim 1, c h a r a c t e r i z e d in that in the consisting isopolypeptide polycation carrier molecules there are monomeres of the same configuration (i.e. either D-, or L-), and the individual monomeres are not linked together by their amino groups in the α-positions, but by those in other (i.e. in β-, γ-, δ-, ε-...etc.) positions - according to the value of "**m**"-, and thus the isopolypeptide polycation carrier molecules (furtheron: carrier molecules), having free α-amino groups, are of general formula (**I/a**):

$$\begin{array}{l} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ \qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\quad NH_2 \\ \qquad\qquad\quad \text{(free α-amino group)} \end{array} \qquad \textbf{(I/a)}$$

wherein

"**r**" and "**m**" have the same meaning as in general formula (**I**).

3) Carrier molecules of general formula (**I/a**), prepared according to Claim 2, c h a r a c t e r i z e d in that their structure is divergent from that of the polypeptides build up by customary α-amino-peptide bonds, generally occurring in mammal organisms, and due to

their divergent chemical structure, for instance the ε-amino-peptide bonds, they also exhibit divergent biological properties.

4) Carrier molecules of general formula (**I/a**), according to Claim 3, characterized in that they are more resistant to proteolytic enzymes, and thus are especially favourably applicable in transporting the active substances of different types within the mammal organism, i.e. for implementing carrier functions.

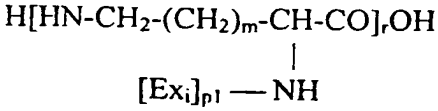
5) Polycation bioconjugates of general formula (**I**), prepared according to Claim 1, characterized in that suitably selected [(k)**M**x] and/or [(i)**M**x] molecules, which may either be identical ones or of (two or more i.e. "**x**") different kind, are conjugated to a given representative of carrier molecules of general formula (**I/a**), by covalent and/or ionic bonds.

6) Polycation bioconjugates of general formula (**I**), according to Claim 5, characterized in that the conjugation of the [(k)**M**x] and/or [(i)**M**x] molecules to a given representative of carrier molecules of general formula (**I/a**), by covalent and/or ionic chemical bonds takes place directly and/or indirectly, in a definite ratio, preferably to reach a saturation of 10 to 100 %.

7) Polycation bioconjugates of general formula (**I**), prepared according to Claim 1, characterized in that they include those bioconjugates in which a given representative of carrier molecules of general formula (**I/a**), is directly conjugated by covalent bonds with [Ex_i] enhancer molecules, which may either be identical ones or of (two or more i.e. "**x**") different kind, and in these bioconjugates:

$$[(k)\mathbf{M}x] = [Ex_i]_{p1},$$

and the polycation bioconjugates are being described by the general formula (**II**):



(II)

wherein:

"**Ex**" in $[Ex_i]_{p1}$ designates the Ex enhancer molecules of different ("**x**") kind conjugated directly to a given representative of carrier molecules of general formula (**I/a**), by covalent bonds, and

"**i**" indicates whether the Ex enhancer molecules, conjugated to the given carrier molecule by covalent bonds, are identical ones (**i** = 1), or they are of different kind, of number "**i**" (**i** = 2, 3,... "**x**" kind); and

"p_1" indicates a degree of saturation in % of a carrier molecule of general formula (**I/a**) with [Ex_i] enhancer molecules, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2 -groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates; and

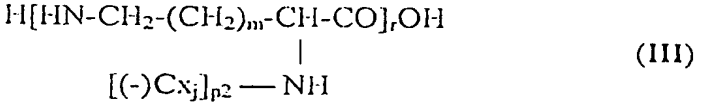
"**r**" and "**m**" have the same meaning as in general formula (**I**).

8) Carrier molecules of general formula (**I/a**), prepared according to Claim 2, characterized in that a given representative of them are conjugated by covalent bonds

with [(-)Cx$_j$] connecting molecules of anionic character, which may either be identical ones or of (two or more i.e. "x") different kind, and the connecting molecules are suitably chosen dicarbonic acids, tricarbonic acids, carbohydrates, or amino acids, or peptide chain elongators, and in these compounds:

$$[(k)Mx] = [(-)Cx_j]_{p2}$$

and the conjugates are being described by the general formula (III):



(III)

wherein:

"(-)Cx" in [(-)Cx$_j$]$_{p2}$ designates (-)Cx connecting molecules of exclusively anionic character of different ("x") kind linked to a given representative of carrier molecules of general formula (I/a) by covalent bonds, and

"j" indicates whether the (-)Cx connecting molecules, conjugated to the given carrier molecule by molecule by covalent bonds, are identical ones (i = 1), or they are of different kind, of number "i" (i = 2, 3, ... "x" kind); and

"p_2" indicates a degree of saturation in % of a carrier molecule of general formula (I/a) by [(-)Cx$_j$] connecting molecules of exclusively anionic character, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2 – groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates; and

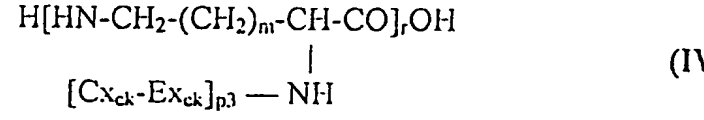
"r" and "m" have the same meaning as in general formula (I).

9) Conjugates of general formula (III), according to Claim 8, characterized in that the carrier molecules of general formula (I/a), of cationic character, due to conjugation of the [(-)Cx$_j$] connecting molecules of anionic character to them by covalent bonds, become capable of building up such polycation bioconjugates, in which additional possibilities arise to establish ionic bonds with enhancer molecules of cationic character.

10) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which a given representative of carrier molecules of general formula (I/a) is indirectly conjugated with Ex enhancer molecules, which may either be identical ones or of (two or more i.e. "x") different kind, through Cx connecting molecules, which may also be either identical ones or of (two or more i.e. "x") different kind, and in these bioconjugates both of the chemical bonds between the carrier molecule and Cx, as well as between the Cx and Ex, are covalent ones, and in these bioconjugates:

$$[(k)Mx] = [Cx_{ck}\text{-}Ex_{ck}]_{p3},$$

and the polycation bioconjugates are being described by the general formula (IV):



(IV)

wherein:

"**Cx-Ex**" in $[Cx_{ck}\text{-}Ex_{ek}]_{p3}$ designates the **Ex** enhancer molecules of different ("**x**") kind, conjugated by covalent bonds indirectly, through **Cx** connecting molecules of different ("**x**") kind, that are also conjugated by covalent bonds to a given representative of carrier molecules of general formula (**I/a**), and

"**ck**" indicates whether the **Cx** connecting molecules, conjugated to the given carrier molecule by covalent bonds, are identical ones (**ck** = 1), or they are of different kind of the number "**ck**" (**ck** = 2, 3, ... "**x**" kind), and

"**ek**" indicates whether the **Ex** enhancer molecules, conjugated to the given carrier molecule indirectly through **Cx** connecting molecules by covalent bonds, are identical ones (**ek** = 1), or they are of different kind of the number "**ek**" (**ek** = 2, 3,... "**x**" kind),

"p_3" means a degree of saturation in % of a carrier molecule by $[Ex_{ek}]$ enhancer molecules coupled to $[Cx_{ck}]$ connecting molecules, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2 -groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates, and further

"**r**" and "**m**" have the same meaning as in general formula (**I**).

11) Conjugates of general formula (**III**), according to Claim 8, characterized in that the $[(-)Cx_j]$ connecting molecules of different ("**x**") kind, occurring in them are suitably chosen dicarbonic acids, tricarbonic acids, carbohydrates, or amino acids, or peptide chain elongators.

12) Polycation bioconjugates of general formula (**I**), according to Claim 1, characterized in that they include those bioconjugates in which to a given representative of carrier molecules of general formula (**I/a**), are conjugated by covalent bonds

a/ $[Ex_i]$ enhancer molecules and/or

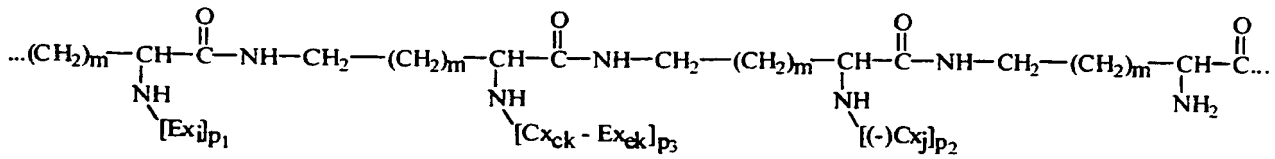
b/ $[(-)Cx_j]$ connecting molecules of anionic character and/or

c/ $[Cx_{ck}\text{-}Ex_{ek}]$ indirectly coupled enhancer molecules

which may either be identical ones or of (two or more i.e. "**x**") different kind, with the proviso, that from among the $[Ex_i]$ and/or $[(-)Cx_j]$ and/or $[Cx_{ck}\text{-}Ex_{ek}]$ types of molecules at least two are contained in the bioconjugate, and in these bioconjugates:

$$[(k)Mx] = [Ex_i]_{p1} + [(-)Cx_j]_{p2}, \text{ or}$$
$$[Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3}, \text{ or}$$
$$[Cx_{ck}\text{-}Ex_{ek}]_{p3} + [(-)Cx_j]_{p2}, \text{ or}$$
$$[Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} + [(-)Cx_j]_{p2},$$

and the polycation bioconjugates are being described by the schematic formula (**V**):



(V)

wherein:

"$[Ex_i]_{p1}$"	has the same meaning as in general formula (**II**),
"$[(-)Cx_j]_{p2}$"	has the same meaning as in general formula (**III**),
"$[Cx_{ck}\text{-}Ex_{ek}]_{p3}$"	has the same meaning as in general formula (**IV**),

"**m**" has the same meaning as in general formula (**I**), further the value of "p_1"+"p_2"+"p_3" > 0 and ≤ 100, and from among "p_1", "p_2" and "p_3" the value of at least two are greater than 0; further in a given polycation bioconjugate, the Ex molecules in $[Ex_i]$, and the (-)Cx molecules in $[(-)Cx_j]$ are not necessarily identical with those Ex and Cx molecules occurring in $[Cx_{ck}\text{-}Ex_{ek}]$ which divergence is symbolized by "**x**".

13) Polycation bioconjugates of general formula (**I**), according to Claim 1, characterized in that they include those bioconjugates in which a given representative of carrier molecules of general formula (**I/a**) is directly conjugated, exclusively by ionic bonds with (-)Ax enhancer molecules of anionic character, which may either be identical ones or of (two or more i.e. "**x**") different kind, and in these bioconjugates

$$[(i)Mx] = [(-)Ax_s]_t,$$

and the polycation bioconjugates so obtained can be described by the general formula (**VI**):

$$H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH(\text{-}[(-)Ax_s]_t\text{-}NH_2)\text{-}CO]_rOH \qquad \textbf{(VI)}$$

wherein:

"(-)Ax" in $[(-)Ax_s]_t$ designates those (-)Ax enhancer molecules of anionic character, which may either be identical ones or of (two or more i.e. "**x**") different kind, that are conjugated to a given representative of carrier molecules of general formula (**I/a**), by ionic bonds, and

"**s**" indicates whether the anionic/polyanionic molecules, conjugated to the given polycation carrier molecule by ionic bonds, are identical ones (s=1), or, they are of different kind, of the number "**s**" (s = 2, 3,..... "**x**" kind); and

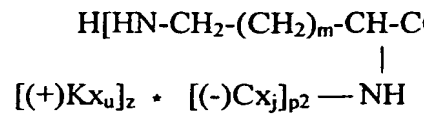
"**t**" means a degree of saturation in % of the given representative of carrier molecules of general formula (**I/a**) by (-)Ax anions, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2-groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates; and

"**r**" and "**m**" have the same meaning as in general formula (**I**).

14) Polycation bioconjugates of general formula (**I**), according to Claim 1, characterized in that they include conjugates of general formula (**III**), prepared according to Claim 8, and these are conjugated with (+)Kx enhancer molecules of cationic character, which may either be identical ones or of (two or more i.e. "**x**") different kind, by ionic bonds via the (-)Cx connecting molecules of anionic character, and in these bioconjugates

$$[(k/i)Mx] = [(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z,$$

and the polycation bioconjugates are being described by the general formula (**VII**):



(VII)

wherein:

"(+)**Kx**" in $[(+)Kx_u]_z$ designates the (+)Kx enhancer molecules of cationic character, which may either be identical ones or of (two or more i.e. "**x**") different kind, that are conjugated to a given representative of conjugates of general formula (**III**), and

"**u**" indicates whether the cations/polycations conjugated to the given compound of general formula (**III**) by ionic bonds, are identical ones (**u** = 1), or they are of different kind of the number "**u**" (**u** = 2, 3,.....i.e. "**x**" kind), and

"**z**" means a degree of saturation in % of the given representative of compounds of general formula (**III**) by $[(+)Kx_u]$ cations, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in peptide bonds) and bound NH_2-groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates, and as the $[(+)Kx_u]$ molecules of cationic character can exclusively be conjugated through the $[(-)Cx_j]$ connecting molecules of anionic character to the compounds of general formula (**III**), therefore

"p_2" = "**z**", further

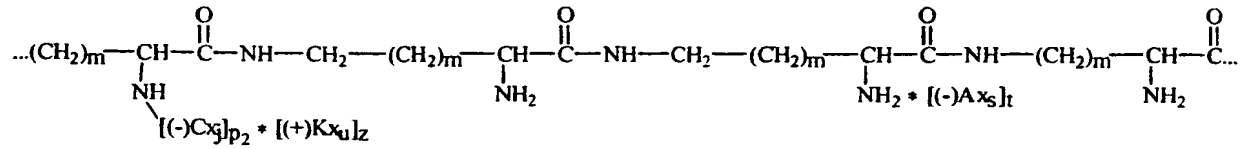
"$[(-)Cx_j]_{p2}$" has the same meaning as in general formula (**III**),

"**r**" and "**m**" have the same meaning as in general formula (**I**).

15) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which to the free α-amino groups of a given representative of polycation bioconjugates of general formula (**VII**), prepared according to Claim 14, as to a polycation, additional enhancer molecules which may either be identical ones or of (two or more i.e. "**x**") different kind, of anionic character $[(-)Ax_s]$ are conjugated, and in these bioconjugates

$$[(k/i)Mx] = \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\} \cdot [(-)Ax_s]_t,$$

and the polycation bioconjugates are being described by the schematic formula (**VIII**):



(VIII)

wherein:

"$[(-)Cx_j]_{p2}$" has the same meaning as in general formula (**III**),

"$[(+)Kx_u]_z$" has the same meaning as in general formula (**VII**),

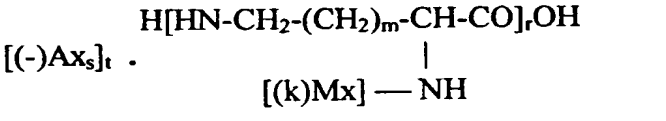
"$[(-)Ax_s]_t$" has the same meaning as in general formula (**VI**),

"**m**" has the same meaning as in general formula (**I**).

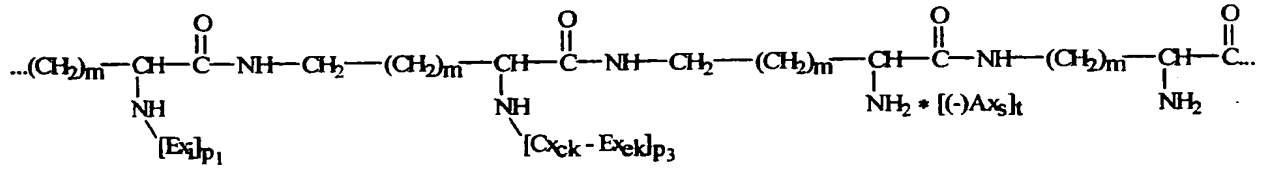
16) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which to the free α-amino groups of a given representative of polycation bioconjugates of general formulae (II), (IV), or of schematic formula (V), prepared according to Claims 7, 10, and 12, as to a polycation, additional enhancer molecules which may either be identical ones (two or more i.e. "x") different kind, of anionic character $[(-)Ax_s]$ are conjugated by ionic bonds, and in these bioconjugates

$$[(k/i)Mx] = [Ex_i]_{p1} * [(-)Ax_s]_t \text{ or } [Cx_{ck}\text{-}Ex_{ek}]_{p3} * [(-)Ax_s]_t \text{ or } [Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} * [(-)Ax_s]_t,$$

and the polycation bioconjugates are being described by the general formula (IX), or by the schematic formula (IX/a):



(IX)



(IX/a)

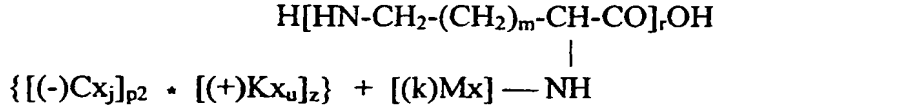
wherein:

"$[(-)Ax_s]_t$"	has the same meaning as in general formula (VI),
"$[Ex_i]_{p1}$"	has the same meaning as in general formula (II),
"$[Cx_{ck}\text{-}Ex_{ek}]_{p3}$"	has the same meaning as in general formula (IV),
"[(k)Mx]" and "m"	have the same meaning as in general formula (I), further
"p_1"+"p_2"+"t"	> 0 and ≤ 100, and from among
"p_1" and "p_3"	the value of at least one > 0; and
"t" > 0.	

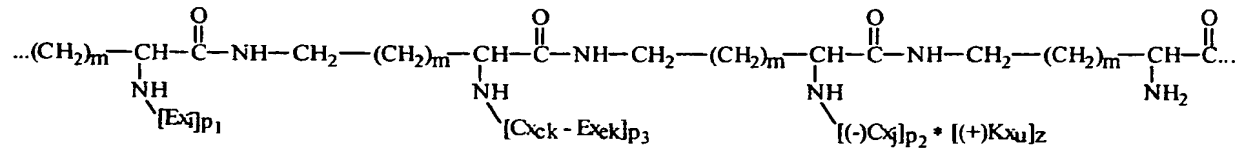
17) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates which correspond to the polycation bioconjugates schematic formula (V), prepared according to Claim 12, including further those bioconjugates in which there are connecting molecules of anionic character $[(-)Cx_j]$ which may either be identical ones or of two or more "x" different kind, and these connecting molecules conjugate with ionic bonds the enhancer molecules of cationic character $[(+)Kx_u]$ which may either be identical ones or of (two or more i.e. "x") different kind, and in these bioconjugates

$$\begin{aligned}[(k/i)Mx] = {} & [Ex_i]_{p1} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} \text{ or} \\ & [Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\}, \text{ or} \\ & [Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\}\end{aligned}$$

and the polycation bioconjugates are being described by the general formula (X), or by the schematic formula (X/a):



(X)

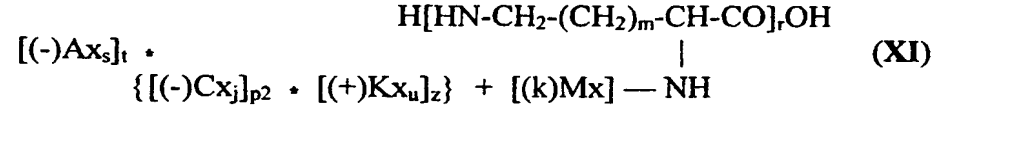


(X/a)

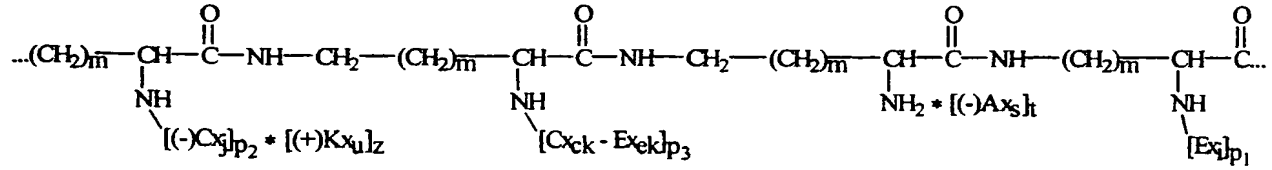
18) Polycation bioconjugates of general formula (**I**), according to Claim 1, c h a r a c t e r i z e d in that they include those bioconjugates in which to the free α-amino groups of a given representative of polycation bioconjugates of general formula (**X**), or of schematic formula (**X/a**), prepared according to Claim 17, as to a polycation, additional enhancer molecules of anionic character [(-)Ax_s] which may either be identical ones or of (two or more i.e. "**x**") different kind, are conjugated by ionic bonds, and in these bioconjugates

$[(k/i)Mx] = [Ex_i]_{p1} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} * [(-)Ax_s]_t$ or
$[Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} * [(-)Ax_s]_t$ or
$[Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} * [(+)Kx_u]_z\} * [(-)Ax_s]_t$,

and the polycation bioconjugates are being described by the general formula (**XI**), or by the schematic formula (**XI/a**):



(XI)



(XI/a)

19) Carrier molecules of general formula (**I/a**), prepared according to Claim 2, having free α-amino groups, c h a r a c t e r i z e d in that they include hydrobromide salts of those 60 – 120 membered, non-racemic polyiso-L-lysines, i.e. poly-(ε)-L-lysine-hydrogen-bromides which themselves possess certain antitumor, antiproliferative and antiviral effect.

20) Carrier molecules of general formula (**I/a**), according to Claim 19, c h a r a c t e r i z e d in that they increase the antiproliferative or antiviral activities of the molecules linked to them.

21) Polycation bioconjugates, according to Claim 1, characterized in that in case the enhancer molecules conjugated to the suitably selected carrier molecule of general formula (**I/a**) themselves are not ab ovo possessing the wanted (eg. antiproliferative) activity, then as a consequence of their conjugation, they will boost the originally existing biological activity of the carrier molecule, for example of those prepared according to Claim 19.

22) Polycation bioconjugates of general formula (I), prepared according to Claim 1, characterized in that each of them contains carrier molecules of general formula (**I/a**) and these suitably selected carrier molecules are conjugated in a manner as shown on the general formulae (**II**), (**III**), (**IV**), (**VI**), (**VII**), (**IX**), (**X**), (**XI**), or on the schematic formulae (**V**), (**VIII**), (**IX/a**), (**X/a**), (**XI/a**), according to Claims 7, 8, 10, 12, 13, 14, 15, 16, 17 and 18, with practically any organic and/or inorganic molecule possessing functional groups appropriate for conjugation, and these latter may rationally be chosen - with a non-limiting manner – from among the groups of compounds, set out hereinbelow:

- hormones and hormone antagonists of different kind (steroid, protein, peptide, etc.), and active fragments of peptide hormones, and derivatives thereof;
- saturated and unsaturated fatty acids, cholesterols, phospholipides (phosphoglycerides, sphingomyelins, etc.), and derivatives thereof;
- nucleic acids/antisense nucleotides;
- monosaccharides, oligosaccharides, and polysaccharides, and derivatives thereof;
- vitamines, and their derivatives;
- known antitumor drugs and active substances, and derivatives thereof;
- amino acids, oligopeptides, polypeptides, further glycoproteins and lipoproteins, their fragments, and derivatives thereof.

23) Polycation bioconjugates of the general formula (I), according to Claim 1, characterized in that each of them contains carrier molecules of general formula (**I/a**), which are conjugated directly and/or indirectly, by covalent and/ionic bond, with enhancer molecules having direct antiproliferative effect, which may either be identical ones or of (two or more i.e. "**x**") different kind, and these polycation bioconjugates are successfully applicable for treating malignant tumors occurring in mammal organisms, *in se*, or in combination with known antitumor methods, accepted in clinical practice.

24) Polycation bioconjugates of the general formula (I), according to Claim 23, characterized in that each of them contains carrier molecules of general formula (**I/a**), which are conjugated with additional enhancer molecules having indirect antiproliferative effect that is developing selectivity or increasing selectivity of bioconjugates towards a given target tumor cell, whereby they inrease the concentration of the bioconjugates in the tumors, and thus the unwanted side-effects can be diminished, and the effectiveness of the treatment may further be increased.

25) Polycation bioconjugates of general formula (I), according to Claims 23 and 24, characterized in that in order to endow them with antitumor effect, a given representative of carrier molecules of general formula (**I/a**), is conjugated with compounds, suitably selected, from among the molecules having direct and/or indirect antiproliferative activity as demonstrated hereinbelow - by a non-limiting manner:

- compounds having direct antiproliferative effects: cytostatics used in the clinical practice, furthermore cytokines, which influence division and differentiation of tumor cells (eg.

different growth factors as well as antibodies produced against them, interferons, etc.), furthermore peptides/proteins inhibiting formation of new blood-vessels around the tumor cells (angiostatins, endostatins), further nucleic acids/antisense oligonucleotides which exert antiproliferative effects on the malignantly transformed cells;

- compounds of indirect antiproliferative effect, which develop selectivity or increase the selectivity of bioconjugates towards a given target cell: monoclonal antibodies having specific affinities to a surface antigen of a given tumor cell, as well as antibodies or any compound having affinity to those kind of receptors (for example transferrin receptor or folate receptor among the vitamins, etc.) which are present in a greater ratio on the surface of the tumor cells than of the normal (not malignantly transformed) cells;
- compounds having direct and simultaneously indirect antiproliferative effects: hormones, hormone antagonists and derivatives thereof, especially from among the polypeptide hormones the humane choriogonadotropine hormone, which having antiproliferative effects, furthermore antibodies produced against receptors of growth factors of different kind, which are present in greater ratio on the surface of a given tumor cell than on other cells, and simultaneously exert antiproliferative effects towards given malignantly transfomed cells, furthermore immunotoxines, which are produced against a given tumor cell;

26) Polycation bioconjugates of general formula (**I**), according to Claim 1, characterized in that to a suitably selected given representative of the consisting carrier molecules of general formula (**I/a**), as to a polycation, a suitably selected nucleic acid - being a polyanion - is linked by ionic bond, and therefore the synthetized new conjugate is appropriate for gene transfer.

27) Polycation bioconjugates of general formula (**I**), according to Claim 26, characterized in that besides the nucleic acid coupled to the given representative of the consisting carrier molecules of general formula (**I/a**) by ionic bonds, additional molecules, which may either be identical ones or of two or more "**x**" different kind, capable of binding selectively and/or semi-selectively to target cells picked out for gene transfer, such as specific antibodies, hormones and molecules binding only to receptors on the surface of the target cells, which for example occur – due to non-pathological changes – exclusively on some cell types, namely to asialoglycoprotein receptor on liver cells (to which galactose residues in terminal position on the macromolecules are specifically binding to) or those molecules which are binding to receptors that occur more frequently (in more %) on the given target cells, are conjugated by covalent and/or ionic bonds, whereby a targeted gene transfer on cellular level is accomplished by the new conjugates.

28) Polycation bioconjugates of general formula (**I**), according to Claim 26 and Claim 27, characterized in that the bioconjugates, that are appropriate for gene transfer, consisting suitably selected isolated or synthetized nucleic acids, complexed antisense oligonucleotides, exhibit antiproliferative, antimicrobial effect, whereby for example inhibition of virus replication can be achieved, further they are enabling the treatment of genetic diseases (eg. cystic fibrosis).

29) Polycation bioconjugates of general formula (**I**), according to Claim 1, characterized in that to suitably selected given representatives of consisting carrier molecules of general formula (**I/a**) (which themselves possess certain antiviral activity) compounds having antimicrobial effect, (eg. antiviral, antibacterial, antimycotic, anti-protozoic compounds used in clinical practice, further complex antisense nucleic acids inhibiting

replication of viruses, antibodies of neutralizing effect) are conjugated, whereby the antimicrobial effect of the conjugated molecules increases.

30) Polycation bioconjugates of general formula (I), according to Claim 29, characterized in that besides the compounds of antimicrobial activity, conjugated to a given representative of carrier molecules of general formula (I/a), additional molecules, which may either be identical ones or of two or more "x" different kind, capable of binding selectively to microbes, such as specific antibodies that link to them, are conjugated, whereby the antimicrobial effect is further increasing.

31) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that to a given representative of consisting carrier molecules of general formula (I/a), paramagnetic contrast material, for example complex derivatives of gadolinium, is conjugated by means of chemical bonds, whereby the diagnostic value of nuclear magnetic resonance (NMR) imaging improves as compared to that of non-conjugated paramagnetic contrast material.

32) Polycation bioconjugates of general formula (I), according to Claim 31, characterized in that besides the molecules of the paramagnetic contrast material, conjugated to a given representative of included carrier molecules of general formula (I/a), they also include further suitably selected molecules - which may either be identical ones or of two or more "x" different kind – also conjugated, which are capable of being selectively enriched in organs, tissues, or pathological changes (eg. tumors) to be investigated, and the increase of their relative concentration within the given target area improves the image quality, additionally.

33) Polycation bioconjugates of general formula (I), according to Claims 31 and 32, characterized in that the molecules enhancing selectivity, which are conjugated to a given representative of the carrier molecules of general formula (I/a), are comprising antibodies, binding specifically to the target area to be investigated by NMR, or depending on the nature of the target area, also lipophilic or hydrophilic substances.

34) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they are being introduced into the target organism on transdermal route via iontophoresis.

35) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they are capable of being appropriately incorporated into liposomes.

36) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that the regulating effect of the immune response modifying molecules, for example of the lymphokines (interleukines, interferons, etc.), that are conjugated to a given representative of carrier molecules of the general formula (I/a), as a constituent of the above polycation bioconjugates, is coming more favourably to full display.

37) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that these are being formulated in pharmaceutically acceptable forms, and the pharmaceutical preparates so obtained, are applicable perorally, or parenterally, or transdermally, for systemic or topical use.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 April 2001 (06.04.01)	
International application No. PCT/HU00/00061	**Applicant's or agent's file reference** "BIOCONJUGATE"
International filing date (day/month/year) 28 June 2000 (28.06.00)	**Priority date** (day/month/year) 29 June 1999 (29.06.99)
Applicant SZEGŐ, Péter	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   25 January 2001 (25.01.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Henrik Nyberg
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

14

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference "BIOCONJUGATE"	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/HU00/00061	International filing date *(day/month/year)* 28/06/2000	Priority date *(day/month/year)* 29/06/1999
International Patent Classification (IPC) or national classification and IPC A61K47/48		
Applicant SZEGO, Péter		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/01/2001	24.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Vogt, T Telephone No. +49 89 2399 8477

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/HU00/00061

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-22 as originally filed

Claims, No.:

1-24 with telefax of 09/08/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16, 19-24
	No:	Claims	17, 18
Inventive step (IS)	Yes:	Claims	1-16, 19-24
	No:	Claims	17, 18
Industrial applicability (IA)	Yes:	Claims	1-16, 19-24
	No:	Claims	17, 18

2. Citations and explanations
see separate sheet

I Amendments (Art. 41 PCT).

The applicant deleted claims 3, 4, 11 19, 20, 22 and 25 as originally filed.
Claims 17 and 18 as filed with the letter of 09.08.2001 are according to said letter, respectively, based on claims 23, 28, 29 and 36 and claims 24, 27, 30, 32 and 33 as originally filed.

New claims 17 and 18 do not meet the requirements of Art.41 PCT because they generalize the subject matter beyond the scope of the application as originally filed.

Furthermore, the scope of said claims is rendered unclear, because the scope of the claims is defined by the result to be achieved (Art. 6 PCT).

V Reasoned Statement (Rule 66(2) PCT).

Subject matter of the present application.

Conjugates between a carrier and an active compound, wherein the carrier is a polymer of a cationic amino acid derivative (eg. Lys) wherein the polymer is formed between the carboxyl group and the β, γ, δ, ε-NH group (instead of the α-NH group as in polypeptides) and the active compound is bound/complexed to the free α-NH group.

Cited prior art documents (Rule 64(1) PCT).

D1: DE-A-3835962.

D2: UCHEGBU ET AL. (1998) PROC. INT. SYMP. CONTROLLED RELEASE BIOACT. MATER. 25TH, 186-187.

D3: WANG ET AL. (2000) PROC. INT. SYMP. CONTROLLED RELEASE BIOACT. MATER. 27TH, 367-368.

Novelty (Art. 33(2) PCT).

The difference with the cited prior art (not limited to the three documents listed above but also those cited on p. 10-14 of the description) is that not poly-α-L-Lys is used as a carrier but, for instance, poly-ε-L-Lys. The advantage of this simple modification is that the resulting molecule is more resistant against proteases.

D1 (cited in the description as HU-B-202553) discloses the chemical preparation of a preferred carrier (poly-ε-L-Lys, cf. p. 9 5th paragraph and example 1 of the present

application). D1 furthermore discloses that poly-ε-L-Lys is produced by Streptomyces albulus and inactivates bacteriophages. D1 anticipates the use of poly-ε-L-Lys in therapeutic compositions.

D2 and D3 relate to poly-α-L-Lys conjugates.

The subject matter of the present application is novel (Art. 33(2) PCT).

Inventive step (Art. 33(3) PCT).

Poly-α-L-Lys is known from the prior art as a carrier for therapeutic compounds (cf. the long list of references on p. 10-14 of the present application). In contrast the applicant states in the letter of reply that poly-ε-L-Lys of D1 is known to be highly cardiotoxic. The use of poly-ε-L-Lys as a substitute for poly-α-L-Lys would therefore not be obvious.

The applicant has shown that the poly-ε-L-Lys comprising conjugates are unexpectedly non-toxic in in vivo experiments.

Despite the fact that the field of drug targetting is at present developing very rapidly, the applicant is the first to consider that poly-ε-L-Lys like polymers can succesfully substitute for poly-α-L-Lys in medicins.

Although the protection sought by the applicant comprises virtually **all uses** of poly-ε-L-Lys like polymers, the applicant of the present application is the first to make the link with the numerous known uses of poly-α-L-Lys, and there appears to be no reason to believe that any of the applications known for poly-α-L-Lys would not work for poly-ε-L-Lys.

The examiner acknowledges, therefore, the presence of an inventive step for the subject matter of the present application (Art. 33(3) PCT).

Upon entry into the regional phase, however, the applicant should expect to be asked by the examiner to provide experimental evidence for the broad scope of the claims and to provide comparitive tests indicating the toxic nature of the unsubstituted poly-ε-L-Lys.

Industrial applicability (Art. 33(4) PCT).

The conjugates of the present application can be used for the preparation of medicaments and in therapeutic compositions.

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Published:
— *with international search report*

(88) Date of publication of the international search report:
22 November 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYCATION-BASED BIOCONJUGATES

$$H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \quad (I)$$
$$\qquad\qquad\qquad\qquad\qquad\quad |$$
$$[(i)Mx] \bullet [(k)Mx] \text{ —— } NH$$

(57) Abstract: The invention relates to new polycation bioconjugates and to a method for producing them. New polycation bioconjugates according to the invention are characterized by that the comprised polycations are capable of transporting active substances of different types in the mammal organism, i.e. for functioning as carrier molecules, thus are able to enhance the biological effectiveness of the transported molecules, and consequently they can, for example, favourably inhibit malignant cell proliferation, or they possess antimicrobial effect, or are suitable for transportation of genes. A further characteristic of the polycation bioconjugates according to the invention is that each of them contains isopolypeptide carrier molecules, bearing free α-amino group, as a common characteristic structural element. Enhancer molecules - same or different - having appropriate binding functions are coupled by chemical bonds directly and/or indirectly through connecting molecules - that may be identical or different ones - to the carrier molecule. Hence the polycation bioconjugates synthetized according to the invention are of general formula (I) wherein: "r" is a mean value between 20 and 400, "m"=0, 1, 2, 3, ...k, "[(k)Mx]" designates enhancer molecules and/or connecting molecules conjugated by covalent (=k) bonds to the isopolypeptide polycation carrier molecule, and "[(i)Mx]" designates enhancer molecules conjugated by ionic (=i) bonds to the isopolypeptide polycation carrier molecule, whereas the said enhancer molecules and connecting molecules having appropriate functional groups for conjugation may either be identical ones or of (two or more i.e "x") different kinds, and the enhancer molecules can be conjugated directly and/or indirectly through a connecting molecule.

WO 01/00242 A3

INTERNATIONAL SEARCH REPORT

Inter[illegible]al Application No
PCT/HU 00/00061

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EMBASE, BIOSIS, CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DE 38 35 962 A (MTA KUTATAS ES SZERVEZETELEMZO ;NOEVENYVEDELMI KUTATO INTEZET (HU)) 1 June 1989 (1989-06-01) cited in the application page 7, line 54 - line 67 & HU 202 553 B 28 March 1991 (1991-03-28)	1-37
X	UCHEGBU, I. F. ET AL: "Polymeric vesicles from amino acid homopolymers" PROC. INT. SYMP. CONTROLLED RELEASE BIOACT. MATER. (1998), 25TH, 186-187 , XP000993384	1
Y	abstract	1-37

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 May 2001	25/05/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Berte, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/HU 00/00061

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WANG, W. ET AL: "Polymeric vesicle prepared from poly-L-lysine modified amphiphilic graft copolymer" PROC. INT. SYMP. CONTROLLED RELEASE BIOACT. MATER. (2000), 27TH, 367-368 , XP000993385	1
Y	abstract	1-37

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 37 relate to an extremely large number of possible compounds. In fact, the claims contain so many options or variables , that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely the examples.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/HU 00/00061

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 3835962 A	01-06-1989	HU 48279 A	29-05-1989
		CN 1033633 A	05-07-1989
		DK 585988 A	22-04-1989
		ES 2012559 A	01-04-1990
		FI 884890 A	22-04-1989
		FR 2622195 A	28-04-1989
		GB 2212810 A	02-08-1989
		IT 1230584 B	28-10-1991
		JP 2124861 A	14-05-1990
		NL 8802604 A	16-05-1989
		SE 8803765 A	21-10-1988

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Adam Szentpéteri, Jr.
S.B.G. & K. Patent & Law Offices
Andrassy ut. 113
H-1062 Budapest
HONGRIE

PCT COPY

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	30.04.2002 17
Applicant's or agent's file reference "BIOCONJUGATE"	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/HU00/00061	28/06/2000	29/06/1999

Applicant
SZEGO, Péter

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the International application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Gallego, A Tel.+49 89 2399-8102

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference "BIOCONJUGATE"	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/HU00/00061	International filing date *(day/month/year)* 28/06/2000	Priority date *(day/month/year)* 29/06/1999
International Patent Classification (IPC) or national classification and IPC A61K47/48		
Applicant SZEGO, Péter		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/01/2001	31.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Vogt, T Telephone No. +49 89 2399 8477

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/HU00/00061

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-22 as originally filed

Claims, No.:

1-24 with telefax of 09/08/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-16, 19-24
	No:	Claims 17, 18
Inventive step (IS)	Yes:	Claims 1-16, 19-24
	No:	Claims 17, 18
Industrial applicability (IA)	Yes:	Claims 1-16, 19-24
	No:	Claims 17, 18

2. Citations and explanations
see separate sheet

I Amendments (Art. 41 PCT).

The applicant deleted claims 3, 4, 11 19, 20, 22 and 25 as originally filed.
Claims 17 and 18 as filed with the letter of 09.08.2001 are according to said letter, respectively, based on claims 23, 28, 29 and 36 and claims 24, 27, 30, 32 and 33 as originally filed.

New claims 17 and 18 do not meet the requirements of Art.41 PCT because they generalize the subject matter beyond the scope of the application as originally filed.

Furthermore, the scope of said claims is rendered unclear, because the scope of the claims is defined by the result to be achieved (Art. 6 PCT).

V Reasoned Statement (Rule 66(2) PCT).

Subject matter of the present application.

Conjugates between a carrier and an active compound, wherein the carrier is a polymer of a cationic amino acid derivative (eg. Lys) wherein the polymer is formed between the carboxyl group and the β, γ, δ, ε-NH group (instead of the α-NH group as in polypeptides) and the active compound is bound/complexed to the free α-NH group.

Cited prior art documents (Rule 64(1) PCT).

D1: DE-A-3835962.

D2: UCHEGBU ET AL. (1998) PROC. INT. SYMP. CONTROLLED RELEASE BIOACT. MATER. 25TH, 186-187.

D3: WANG ET AL. (2000) PROC. INT. SYMP. CONTROLLED RELEASE BIOACT. MATER. 27TH, 367-368.

Novelty (Art. 33(2) PCT).

The difference with the cited prior art (not limited to the three documents listed above but also those cited on p. 10-14 of the description) is that not poly-α-L-Lys is used as a carrier but, for instance, poly-ε-L-Lys. The advantage of this simple modification is that the resulting molecule is more resistant against proteases.

D1 (cited in the description as HU-B-202553) discloses the chemical preparation of a preferred carrier (poly-ε-L-Lys, cf. p. 9 5th paragraph and example 1 of the present

application). D1 furthermore discloses that poly-ε-L-Lys is produced by Streptomyces albulus and inactivates bacteriophages. D1 anticipates the use of poly-ε-L-Lys in therapeutic compositions.

D2 and D3 relate to poly-α-L-Lys conjugates.

The subject matter of the present application is novel (Art. 33(2) PCT).

Inventive step (Art. 33(3) PCT).

Poly-α-L-Lys is known from the prior art as a carrier for therapeutic compounds (cf. the long list of references on p. 10-14 of the present application). In contrast the applicant states in the letter of reply that poly-ε-L-Lys of D1 is known to be highly cardiotoxic. The use of poly-ε-L-Lys as a substitute for poly-α-L-Lys would therefore not be obvious.

The applicant has shown that the poly-ε-L-Lys comprising conjugates are unexpectedly non-toxic in in vivo experiments.

Despite the fact that the field of drug targetting is at present developing very rapidly, the applicant is the first to consider that poly-ε-L-Lys like polymers can succesfully substitute for poly-α-L-Lys in medicins.

Although the protection sought by the applicant comprises virtually **all uses** of poly-ε-L-Lys like polymers, the applicant of the present application is the first to make the link with the numerous known uses of poly-α-L-Lys, and there appears to be no reason to believe that any of the applications known for poly-α-L-Lys would not work for poly-ε-L-Lys.

The examiner acknowledges, therefore, the presence of an inventive step for the subject matter of the present application (Art. 33(3) PCT).

Upon entry into the regional phase, however, the applicant should expect to be asked by the examiner to provide experimental evidence for the broad scope of the claims and to provide comparitive tests indicating the toxic nature of the unsubstituted poly-ε-L-Lys.

Industrial applicability (Art. 33(4) PCT).

The conjugates of the present application can be used for the preparation of medicaments and in therapeutic compositions.

1) Polycation bioconjugates characterized in that each of them contains isopolypeptide polycation carrier molecules having free α-amino groups, and these carrier molecules are conjugated by chemical bonds with suitably selected molecules which may eith r be identical ones or of (two or more i.e. "x") different kind, bearing functional groups appropriate for conjugation, and the polycation bioconjugates synthetized this way can be described by the general formula (I):

$$\begin{array}{l} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ \qquad\qquad\qquad\qquad\quad | \\ [(i)Mx] \cdot [(k)Mx] \text{ — } NH \end{array} \qquad \textbf{(I)}$$

wherein:

"r" designates the number of diamino-monocarbonic acyl group monomers which is between 20 and 400 as a mean value,

"m" = 0, 1, 2, 3, ... k,

"[(k)Mx]" designates enhancer molecules and/or connecting molecules conjugated by covalent (= k) bonds to the isopolypeptide polycation carrier molecule, and

"[(i)Mx]" designates enhancer molecules conjugated by ionic (=i) bonds to the isopolypeptid polycation carrier molecule, whereas the said enhancer molecules and connecting molecules having appropriate functional groups for conjugation may either be identical ones or of (two or more i.e. "x") different kind, and the enhancer molecules can be conjugated:

- directly and/or
- indirectly through a connecting molecule,

and further the joint occurrence of [(k)Mx] and [(i)Mx] within the same polycation bioconjugate is symbolized by [(k/i)Mx].

2) Polycation bioconjugates of general formula (I), prepared according to Claim 1, characterized in that the isopolypeptide polycation carrier molecules, which are included in each of polycation bioconjugates, synthetised from diamino-monocarbonic acyl group monomers, are of the same configuration (i.e. either D-, or L-), and the individual monomers are not linked together by their amino groups in the α-positions, but by those in other (i.e. in β-, γ-, δ-, ε-...etc.) positions - according to the value of "m"-, and thus the isopolypeptide polycation carrier molecules (further on: carrier molecules), having free α-amino groups, are of general formula (I/a):

$$\begin{array}{l} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ \qquad\qquad\qquad\quad | \\ \qquad\qquad\quad NH_2 \\ \qquad\quad \text{(free α-amino group)} \end{array} \qquad \textbf{(I/a)}$$

wherein

"r" and "m" have the same meaning as in general formula (I).

3) Polycation bioconjugates of general formula (I), prepared according to Claim 1, characterized in that suitably selected [(k)Mx] and/or [(i)Mx] molecules, which may either be identical ones or of (two or more i.e. "x") different kind, are conjugated to a given representative of carrier molecules of general formula (I/a), by covalent and/or ionic bonds.

4) Polycation bioconjugates of general formula (I), according to Claim 3, characterized in that the conjugation of the [(k)Mx] and/or [(i)Mx] molecules to a given representative of carrier molecules of general formula (I/a), by covalent and/or ionic bonds takes place directly and/or indirectly in a definite ratio, preferably t reach a saturation of 10 to 100 %.

5) Polycation bioconjugates of general formula (I), prepared according to Claim 1, characterized in that they include those bioconjugates in which a given representative of carrier molecules of general formula (I/a) is directly conjugated by covalent bonds with $[Ex_i]$ enhancer molecules, which may either be identical ones or of (two or more i.e. "x") different kind, and in these bioconjugates:

$$[(k)Mx] = [Ex_i]_{p1},$$

and the polycation bioconjugates are being described by the general formula (II):

$$\begin{array}{c} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ | \\ [Ex_i]_{p1} — NH \end{array} \qquad (II)$$

wherein:

"Ex" in $[Ex_i]_{p1}$ designates the Ex enhancer molecules of different ("x") kind conjugated directly to a given representative of carrier molecules of general formula (I/a) by covalent bonds, and

"i" indicates whether the Ex enhancer molecules, conjugated to the given carrier molecule by covalent bonds, are identical ones (i = 1), or they are of different kind according to the number "i" (i = 2, 3,… "x"), and

"p_1" indicates a degree of saturation in % of a carrier molecule of general formula (I/a) with $[Ex_i]$ enhancer molecules, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2-groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates, and

"r" and "m" have the same meaning as in general formula (I).

6) Carrier molecules of general formula (I/a), prepared according to Claim 2, characterized in that a given representative of them are conjugated by covalent bonds with $[(-)Cx_j]$ connecting molecules of anionic character, which may either be identical ones or of (two or more i.e. "x") different kind, and the connecting molecules are suitably chosen dicarbonic acids, tricarbonic acids, carbohydrates, or amino acids, or peptide chain elongators, and in these compounds:

$$[(k)Mx] = [(-)Cx_j]_{p2}$$

and the conjugates are being described by the general formula (III):

$$\begin{array}{c} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ | \\ [(-)Cx_j]_{p2} — NH \end{array} \qquad (III)$$

wherein:

"(-)Cx" in $[(-)Cx_j]_{p2}$ designates (-)Cx connecting molecules of exclusively anionic character of different ("x") kind linked to a given representative of carrier molecules of general formula (I/a) by covalent bonds, and

"j" indicates whether the (-)Cx connecting molecules, conjugated to the given carrier molecule by covalent bonds, are identical ones (j = 1), or they are of different kind according to the number " j " (j = 2, 3, ... "x"), and

"p_2" indicates a degree of saturation in % of a carrier molecule of general formula (I/a) by $[(-)Cx_j]$ connecting molecules of exclusively anionic character, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2 – groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates, and

"r" and "m" have the same meaning as in general formula (I).

7) Conjugates of general formula (III), according to Claim 6, characterized in that the carrier molecules of general formula (I/a) of cationic character, due to conjugation of the $[(-)Cx_j]$ connecting molecules of anionic character to them by covalent bonds, become capable of building up such polycation bioconjugates, in which additional possibilities arise to establish ionic bonds with enhancer molecules of cationic character.

8) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which a given representative of carrier molecules of general formula (I/a) is indirectly conjugated with $[Ex_{ek}]$ enhancer molecules, which may either be identical ones or of (two or more i.e. "x") different kind, through $[Cx_{ck}]$ connecting molecules, which may also be either identical ones or of (two or more i.e. "x") different kind, and in these bioconjugates both of the chemical bonds between the carrier molecule and $[Cx_{ck}]$, as well as between the $[Cx_{ck}]$ and $[Ex_{ek}]$ are covalent ones, and in these bioconjugates:

$$[(k)Mx] = [Cx_{ck}\text{-}Ex_{ek}]_{p3},$$

and the polycation bioconjugates are being described by the general formula (IV):

$$\begin{array}{c} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}\underset{\displaystyle |}{CH}\text{-}CO]_rOH \\ [Cx_{ck}\text{-}Ex_{ek}]_{p3} - NH \end{array} \qquad \textbf{(IV)}$$

wherein:

"Cx-Ex" in $[Cx_{ck}\text{-}Ex_{ek}]_{p3}$ designates the Ex enhancer molecules of different ("x") kind, conjugated by covalent bonds indirectly, through Cx connecting molecules of different ("x") kind, that are also conjugated by covalent bonds to a given representative of carrier molecules of general formula (I/a), and

"ck" indicates whether the Cx connecting molecules, conjugated to the given carrier molecule by covalent bonds, are identical ones (ck = 1), or they are of different kind according to the number "ck" (ck = 2, 3, ... "x"), and

"ek" indicates whether the Ex enhancer molecules, conjugated to the given carrier molecule indirectly through Cx connecting molecules by covalent bonds, are identical ones (ek = 1), or they are of different kind according to the number "ek" (ek = 2, 3,... "x"),

"p_3" means a degree of saturation in % of a carrier molecule by $[Ex_{ek}]$ enhancer molecules coupled to $[Cx_{ck}]$ connecting molecules, the value of which is > 0 and ≤ 100, whereby the

ratio between the free (not involved in chemical bonds) and bound NH_2 -groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates, and further

"r" and "m" have the same meaning as in general formula (I).

9) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which to a given representative of carrier molecules of general formula (I/a), are conjugated by covalent bonds

a/ $[Ex_i]$ enhancer molecules and/or

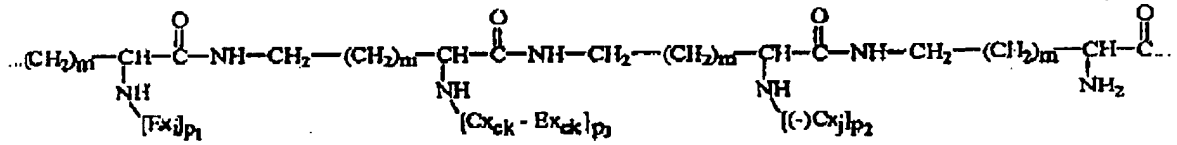
b/ $[(-)Cx_j]$ connecting molecules of anionic character and/or

c/ $[Cx_{ck}\text{-}Ex_{ek}]$ indirectly coupled enhancer molecules

which may either be identical ones or of (two or more i.e. "x") different kind, with the proviso, that from among the $[Ex_i]$ and/or $[(-)Cx_j]$ and/or $[Cx_{ck}\text{-}Ex_{ek}]$ types of molecules at least two ar contained in the bioconjugate, and in these bioconjugates:

$$[(k)Mx] = [Ex_i]_{p1} + [(-)Cx_j]_{p2}, \text{ or}$$
$$[Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3}, \text{ or}$$
$$[Cx_{ck}\text{-}Ex_{ek}]_{p3} + [(-)Cx_j]_{p2}, \text{ or}$$
$$[Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} + [(-)Cx_j]_{p2},$$

and the polycation bioconjugates are being described by the schematic formula (V):



(V)

wherein:

"$[Ex_i]_{p1}$"	has the same meaning as in general formula (II),
"$[(-)Cx_j]_{p2}$"	has the same meaning as in general formula (III),
"$[Cx_{ck}\text{-}Ex_{ek}]_{p3}$"	has the same meaning as in general formula (IV),

"m" has the same meaning as in general formula (I), further the value of "p_1"+"p_2"+"p_3" > 0 and ≤ 100, and from among "p_1", "p_2" and "p_3" the value of at least two are greater than 0; further in a given polycation bioconjugate the Ex molecules in $[Ex_i]$, and the (-)Cx molecules in $[(-)Cx_j]$ are not necessarily identical with those Ex and Cx molecules occurring in $[Cx_{ck}\text{-}Ex_{ek}]$, which divergence is symbolized by "x".

10) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which a given representative of carrier molecules of general formula (I/a) is directly conjugated, exclusively by ionic bonds with $[(-)Ax_a]$ enhancer molecules of anionic character, which may either be identical ones or of (two or more i.e. "x") different kind, and in these bioconjugates

$$[(i)Mx] = [(-)Ax_a]_i.$$

and the polycation bioconjugates so obtained can be described by th general formula (VI):

$$\begin{array}{c} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ | \\ [(-)Ax_s]_t \cdot NH_2 \end{array} \qquad (VI)$$

wherein:

"(-)Ax" in $[(-)Ax_s]_t$ designates those (-)Ax enhancer molecules of anionic character, which may either be identical ones or of (two or more i.e. "x") different kind, that are conjugated to a given representative of carrier molecules of general formula (I/a) by ionic bonds, and

"s" indicates whether the anionic/polyanionic molecules, conjugated to the given carrier molecule by ionic bonds, are identical ones (s = 1), or, they are of different kind according to the number "s" (s = 2, 3,..... "x"), and

"t" means a degree of saturation in % of the given representative of carrier molecules of general formula (I/a) by $[(-)Ax_s]$ anions, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2-groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates, and

"r" and "m" have the same meaning as in general formula (I).

11) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include conjugates of general formula (III), prepared according to Claim 6, and these are conjugated with $[(+)Kx_u]$ enhancer molecules of cationic character, which may either be identical ones or of (two or more i.e. "x") different kind, by ionic bonds via the $[(-)Cx_j]$ connecting molecules of anionic character, and in these bioconjugates

$$[(k/i)Mx] = [(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z,$$

and the polycation bioconjugates are being described by the general formula (VII):

$$\begin{array}{c} H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \\ | \\ [(+)Kx_u]_z \cdot [(-)Cx_j]_{p2} \text{---} NH \end{array} \qquad (VII)$$

wherein:

"(+)Kx" in $[(+)Kx_u]_z$ designates the (+)Kx enhancer molecules of cationic character, which may either be identical ones or of (two or more i.e. "x") different kind, that are conjugated to a given representative of conjugates of general formula (III), and

"u" indicates whether the cationic/polycationic molecules linked to the given conjugate of general formula (III) by ionic bonds, are identical ones (u = 1), or they are of different kind according to the number "u" (u = 2, 3,.....i.e. "x"), and

"z" means a degree of saturation in % of the given representative of conjugates of general formula (III) by $[(+)Kx_u]$ cations, the value of which is > 0 and ≤ 100, whereby the ratio between the free (not involved in chemical bonds) and bound NH_2-groups is determined, which in turn influences the charge and the cationic character of the polycation bioconjugates, and as the $[(+)Kx_u]$ molecules of cationic character can exclusively be

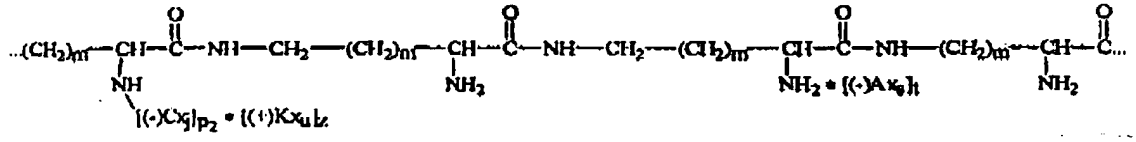
conjugated through the [(-)Cx$_j$] connecting molecules of anionic character to the conjugates of general formula (III), therefore

"p_2" = "z", further
"[(-)Cx$_j$]$_{p2}$" has the same meaning as in general formula (III),
"r" and "m" have the same meaning as in general formula (I).

12) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which to the free α-amino groups of a given representative of polycation bioconjugates of general formula (VII), prepared according to Claim 11, additional [(-)Ax$_s$] enhancer molecules of anionic character which may either be identical ones or of (two or more i.e. "x") different kind, are conjugated by ionic bonds, and in these bioconjugates:

$$[(k/i)Mx] = \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\} \cdot [(-)Ax_s]_t,$$

and the polycation bioconjugates are being described by the schematic formula (VIII):



(VIII)

wherein:

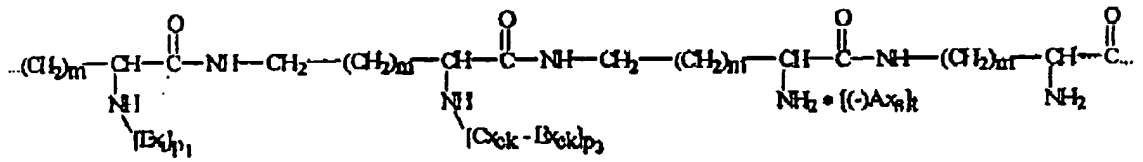
"[(-)Cx$_j$]$_{p2}$" has the same meaning as in general formula (III),
"[(+)Kx$_u$]$_z$" has the same meaning as in general formula (VII),
"[(-)Ax$_s$]$_t$" has the same meaning as in general formula (VI),
"m" has the same meaning as in general formula (I).

13) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which to the free α-amino groups of a given representative of polycation bioconjugates of general formulae (II) or (IV) or of schematic formula (V), prepared according to Claims 5, 8, and 9, additional [(-)Ax$_s$] enhancer molecules of anionic character which may either be identical ones (two or more i.e. "x") different kind are conjugated by ionic bonds, and in these bioconjugates:

$$[(k/i)Mx] = [Ex_i]_{p1} \cdot [(-)Ax_s]_t \text{ or } [Cx_{ck}\text{-}Ex_{ek}]_{p3} \cdot [(-)Ax_s]_t \text{ or } [Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} \cdot [(-)Ax_s]_t,$$

and the polycation bioconjugates are being described by the general formula (IX), or by the schematic formula (IX/a):

$$[(-)Ax_s]_t \cdot H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_r OH \quad \text{(IX)}$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad [(k)Mx] — NH$$



(IX/a)

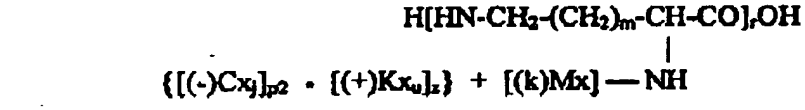
wherein:

"$[(-)Ax_a]_t$"	has the same meaning as in general formula (VI),
"$[Ex_i]_{p1}$"	has the same meaning as in general formula (II),
"$[Cx_{ck}\text{-}Ex_{ek}]_{p3}$"	has the same meaning as in general formula (IV),
"[(k)Mx]", "m", "r"	have the same meaning as in general formula (I), further
"p_1"+"p_3"+"t"	> 0 and ≤ 100, and from among
"p_1" and "p_3"	the value of at least one > 0; and
"t"	> 0.

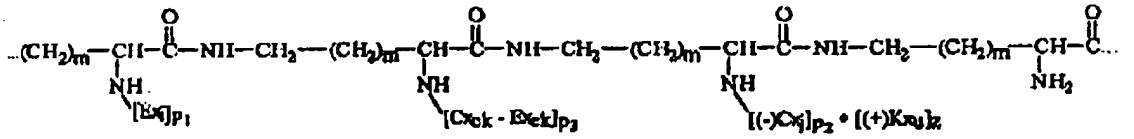
14) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include polycation bioconjugates of schematic formula (V), prepared according to Claim 9, in which there are [(-)Cxj] connecting molecules of anionic character which may either be identical ones or of two or more "x" different kind, and these are conjugated with $[(+)Kx_u]$ enhancer molecules of cationic character which may either be identical ones or of (two or more i.e. "x") different kind, by ionic bonds, and in these bioconjugates

$$[(k/i)Mx] = [Ex_i]_{p1} + \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\} \text{ or}$$
$$[Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\}, \text{ or}$$
$$[Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\}$$

and the polycation bioconjugates are being described by the general formula (X), or by the schematic formula (X/a):



(X)



(X/a)

wherein:

"$[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z$"	has the same meaning as in general formula (VII),
"$[Ex_i]_{p1}$"	has the same meaning as in general formula (II),
"$[Cx_{ck}\text{-}Ex_{ek}]_{p3}$"	has the same meaning as in general formula (IV),
"[(k/i)Mx]", "m", "r"	have the same meaning as in general formula (I), further

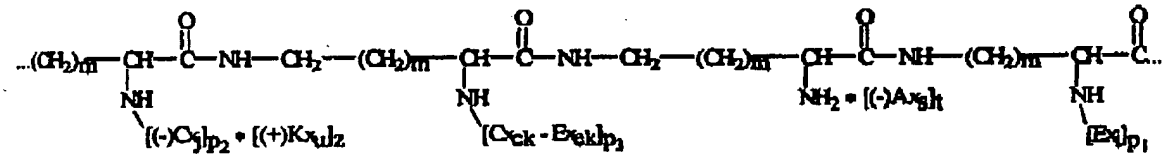
"p_1"+"p_3"+"z"	> 0 and ≤ 100, and from among
"p_1" and "p_3"	the value of at least on > 0; and
"z" = "p_2"	> 0.

15) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they include those bioconjugates in which to the free α-amino groups of a given representative of polycation bioconjugates of general formula (X), or of schematic formula (X/a), prepared according to Claim 14, as to a polycation, additional $[(-)Ax_s]$ enhancer molecules of anionic character which may either be identical ones or of (two or more i.e. "r") different kind, are conjugated by ionic bonds, and in these bioconjugates

$$[(k/i)Mx] = [Ex_i]_{p1} + \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\} \cdot [(-)Ax_s]_t \text{ or}$$
$$[Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\} \cdot [(-)Ax_s]_t \text{ or}$$
$$[Ex_i]_{p1} + [Cx_{ck}\text{-}Ex_{ek}]_{p3} + \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\} \cdot [(-)Ax_s]_t,$$

and the polycation bioconjugates are being described by the general formula (XI), or by the schematic formula (XI/a):

$$[(-)Ax_s]_t \cdot \{[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z\} + [(k)Mx]\text{—}NH\text{—}H[HN\text{-}CH_2\text{-}(CH_2)_m\text{-}CH\text{-}CO]_rOH \quad \text{(XI)}$$



(XI/a)

wherein:

"$[(-)Ax_s]_t$"	has the same meaning as in general formula (VI),
"$[(-)Cx_j]_{p2} \cdot [(+)Kx_u]_z$"	has the same meaning as in general formula (VII),
"$[Ex_i]_{p1}$"	has the same meaning as in general formula (II),
"$[Cx_{ck}\text{-}Ex_{ek}]_{p3}$"	has the same meaning as in general formula (IV),
"[(k/i)Mx]", "m", "r"	have the same meaning as in general formula (I), further
"p_1"+"p_3"+"t"+"z"	> 0 and ≤ 100, and from among
"p_1" and "p_3"	the value of at least one > 0; and
"t"	> 0,
"z" = "p_2"	> 0."

16) Polycation bioconjugates, according to Claim 1, characterized in that in case the enhancer molecules conjugated to the suitably selected carrier molecule of general formula (I/a) themselves are not *ab ovo* possessing the wanted (eg. antiproliferative) activity, then as a consequence of their conjugation, they will boost the originally existing biological activity of the carrier molecule.

17) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that each of them contains carrier molecules of general formula (I/a), which are conjugated directly and/or indirectly, by covalent and/or ionic bonds, with enhancer molecules which may either be identical ones or of (two or more i.e. "x") different kind, having direct therapeutic effects against particular diseases, and the type of the biol gical activities of the polycation bioconjugates is determined by these conjugated enhancer molecules, whereas the bioavailability of the enhancer molecules is increased, due to the conjugation, so the polycation bioconjugates are more successfully applicable for treating such diseases than the particular enhancer molecules alone.

18) Polycation bioconjugates of general formula (I), according to Claim 17 characterized in that further enhancer molecules are additionally conjugated, which may either be identical ones or of (two or more i.e. "x") different kind, having affinity to particular molecules of cells/tissues/organs of the mammal organism, generating this way a selectivity or increasing the selectivity of the polycation bioconjugates towards certain target cells/tissues/organs.

19) Polycation bioconjugates of general formula (I), according to Claims 17 or 18 characterized in that they are more favourably applicable for the treatment of malignant tumors or infections, than the particular unconjugated enhancer molecules alone, from which the polycation bioconjugates are composed of.

20) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that to a suitably selected given representative of the containing carrier molecules of general formula (I/a), as to a polycation, suitably selected nucleic acids – being a polyanion – is linked by ionic bonds, and therefore the polycation bioconjugate is becoming appropriate for gene transfer.

21) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that to a given representative of containing carrier molecules of general formula (I/a), certain paramagnetic contrast materials are conjugated, and additional enhancer molecules, that are capable of being selectively enriched in organs, tissues, or pathological changes (e.g. tumors) to be investigated, whereby the diagnostic value of nuclear magnetic resonance (NMR) imaging is improving.

22) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they are being introduced into the target organism on transdermal route via iontophoresis.

23) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that they are capable of being appropriately incorporated into liposomes.

24) Polycation bioconjugates of general formula (I), according to Claim 1, characterized in that these are being formulated in pharmaceutically acceptable forms, and the pharmaceutical preparates so obtained, are applicable perorally, or parenterally, r transdermally, for systemic or topical use.

PATENT COOPERATION T TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

SZEGO, Péter
Istenhegyi út 88
H-1125 Budapest
HONGRIE

Date of mailing (day/month/year) 03 November 2000 (03.11.00)	
Applicant's or agent's file reference "BIOCONJUGATE"	**IMPORTANT NOTIFICATION**
International application No. PCT/HU00/00061	International filing date (day/month/year) 28 June 2000 (28.06.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 29 June 1999 (29.06.99)
Applicant SZEGO, Péter	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
29 June 1999 (29.06.99)	P 9902217	HU	13 Octo 2000 (13.10.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
Simin Baharlou

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:
VÁCZY, Kristóf
Hegedus Gy. út 8
H-1136 Budapest
HONGRIE

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year)	
04 January 2001 (04.01.01)	
Applicant's or agent's file reference "BIOCONJUGATE"	**IMPORTANT NOTICE**
International application No. PCT/HU00/00061	**International filing date** (day/month/year) 28 June 2000 (28.06.00) / **Priority date** (day/month/year) 29 June 1999 (29.06.99)
Applicant SZEGŐ, Péter	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,DZ,KP,KR,MZ,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD, GE,GH,GM,HR,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO, NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   04 January 2001 (04.01.01) under No. WO 01/00242

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

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1211 Geneva 20, Switzerland

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